DEVELOPMENT OF A NEW TEST SUITE OF ECOLOGICALLY-RELEVANT SPECIES FOR THE ASSESSMENT OF CONTAMINANTS IN BOREAL SOILS – SPECIAL EMPHASIS ON ORIBATID MITES

A Thesis Submitted to the College of Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in the Toxicology Graduate Program
University of Saskatchewan
Saskatoon, Canada

By
Juliska Ilona Princz

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis. Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Director/Chair of the Toxicology Centre
University of Saskatchewan
Saskatoon, Saskatchewan S7N 5A8

ABSTRACT

Boreal regions account for a significant portion of Canada's landmass and economic resources (e.g., forestry, mining, and oil and gas). The inadvertent release of pollutants from industrial activities within these regions stress the need for realistic ecological risk assessments, which can be attained through the use of standardized soil toxicity test methods. Despite the geophysical and economic significance of boreal regions, standardized soil toxicity test methods applicable to these regions are lacking. To help alleviate this gap in ecotoxicology, the objectives of this thesis included the development and application of a new suite of boreal species for the assessment of contaminants in boreal soils. Specifically, research was directed towards the development of a new oribatid mite test, using *Oppia nitens* (C.L. Koch). Oribatid mites represent the most abundant and diverse microarthropod species in forest soils, significantly contributing to nutrient cycling and soil formation processes; however, these organisms are consistently under-represented in soil ecotoxicological assessments. The applicability and limitations of the use of *O. nitens* were demonstrated such that lethal and sublethal endpoints could be derived from the assessment of soils. The performance of *O. nitens* across numerous soils varied such that adult survival was unaffected by soil characteristics, however, reproduction was limited by soil organic matter content.

An evaluation of the sensitivity of *O. nitens*, using a model polycyclic aromatic hydrocarbon was also conducted in a standard test soil. Phenanthrene is a common contaminant in soils, and accumulates within organic-rich horizons, within which oribatid mites live. Therefore, the study was designed to examine the toxicity and bioaccumulation potential of phenanthrene to *O. nitens*, with a comparison of effect to other soil invertebrate species, as presented in the literature. *Oppia nitens* was susceptible to phenanthrene at levels comparable to other soil invertebrate species, and the bioaccumulation of the phenanthrene increased with increasing exposure concentration, although steady-state was not achieved during the four-week exposure duration. The accumulation was biphasic in nature, likely a result of initial cuticular sorption processes, followed by the contribution of other processes (e.g., dietary uptake). The elimination of the phenanthrene was not complete, in that at the end of the elimination phase, tissue residues were similar to that observed in the initial gradual accumulation (e.g., by cuticular

processes). However, the resultant bioaccumulation factors (BAFs) were relatively low, indicative of limited trophic transfer and biomagnification for this species.

Following the development of an *O. nitens* assay, a further study was conducted to compare the sensitivity of O. nitens to other boreal soil invertebrate species, as well as to standard test species. Soil toxicity tests were designed, using field-collected reference and contaminated (petroleum hydrocarbon- and salt-impacted) soils, using an expanded suite of boreal species (plants: *Pinus banksiana*, *Picea glauca*, *Picea mariana*, *Populus tremuloides*, *Calamagrostis canadensis* and *Solidago canadensis*; earthworms: *Dendrodrilus rubidus*; and springtails: *Folsomia nivalis* and *Proisotoma minuta*). The sensitivity of the boreal species was then compared to that of currently published standard soil toxicity test species (agronomic plants: *Elymus lanceolatus* and *Trifolium pratense*; earthworms: *Eisenia andrei*; and springtails: *Folsomia candida*). Estimated species sensitivity distributions (ESSDs) were generated to determine whether the boreal and standard test battery of species exhibited differences in their overall sensitivity to the contaminated soil. With regards to the petroleum-hydrocarbon impacted soil, the suite of boreal species was more sensitive relative to the suite of standard test species; however, upon exposure to the salt-impacted soils, no differences in sensitivity were evident between the boreal and standard suite of test species. In both soils, the performance of *O. nitens* was similar to that observed by the collembolan species. The evaluation of boreal species and soils also took into consideration the use of distinct soil horizons. The layering of horizons was feasible from the initial collection to reassembly for testing in the laboratory, and plant growth was unaffected by this design. However, soil invertebrates demonstrated a preference or avoidance tendency for one horizon over another, and as a result, the assessment of individual horizons was recommended for all future testing. The compilation of research presented herein provides the basis for the standardization of ecologically-relevant test species and methods for the assessment of contaminated soils in boreal regions.

ACKNOWLEDGEMENTS

I would like to sincerely thank my supervisor, Dr. Steven Siciliano, for his guidance and constant encouragement throughout these many years to bring this research to a conclusion. Thanks are also extended to my graduate committee: Dr. Barry Blakley, Dr. Karsten Liber, Dr. Richard Farrell, Dr. Helen Nichol, Dr. Ken van Rees and Dr. Roman Lanno, who served as my external examiner. I'd also like to extend much appreciation to my 'co-supervisor' Rick Scroggins, for without his support, this thesis wouldn't have begun - thank-you for your patience in enabling me to balance work, school, and family throughout this endeavour. Thanks also to Valerie Behan-Pelletier, who was the driving force behind making the use of oribatid mites as a test species possible - her enthusiasm for acarology and science in general is incredible! Sincere gratitude also extends to Gladys Stephenson, who has always pushed me to new limits no matter what life brings. A sincere thank-you is also extended to Mary Moody, not only have you been a major a research partner, but also a great friend.

The research was funded in part by Environment Canada and the Program of Energy Research and Development, through Natural Resources Canada. As a result, I'd like to extend my appreciation to my colleagues at the Biological Assessment and Standardization Section, and in particular, past and current technical staff of the Soil Toxicology Laboratory: H. Lemieux, M. Malette, C. Fraser, E. Ritchie, R. Hennessy, J. Velicogna, L. Van der Vliet, and the many co-op students who have helped in between. Many thanks are extended to Jason Nelson and his team at EcoDynamics Consulting for assistance with soil collections and site characterizations, including insightful conversations about soils, soil conservation and the state of forests in Canada. Worley-Parsons, SHARP Environmental (2000) Ltd., Equilibrium Environmental Inc., and Husky Oil are gratefully acknowledged for site access and assistance with soil collections.

Last, but never least, I am eternally grateful to my family, who are a constant source of encouragement, and to Paul, to whom none of this would ever be possible without your love and support...and a few kiddies thrown in! I dedicate this thesis to my children - Lucia and Sydonia - no dream is ever too small and I hope that you will be encouraged in life, no matter what may come.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A (horizon)	Mineral horizon at or near the soil surface of leaching or eluviation, or accumulation of organic matter (or both) (SCWG, 1998)
AB	Alberta
Ae	A horizon characterized by the eluviation of clay, Al, Fe and/or organic matter
Aegj	Ae horizon further characterized by partial permanent or periodic reduction (indicated by grey colouration)
Ah	A horizon characterized by the accumulation or enrichment of organic matter, but containing <17% organic carbon
Ahe	Ah horizon further characterized by the eluviation of clay, Al, Fe and/or organic matter
Ahg	Ah horizon further characterized by permanent or periodic reduction (grey colouration)
ANOVA	Analysis of variance
AS	Artificial soil
ASE	Accelerated solvent extraction
ASTM	American Society for Testing and Materials
B (horizon)	Mineral horizon characterized by the enrichment of organic matter, sesquioxides or clay, or by the development of soil structure, or by change in colouration denoting hydrolysis, reduction or oxidation (SCWG, 1998)
BAF	Bioaccumulation factor
BaP	Benzo[a]pyrene
BCgj	B and C horizon (mixed together), with the C horizon characterized by partial permanent or periodic reduction (indicated by grey colouration)
Bf	B horizon enriched with amorphous material, mainly Al, Fe combined with organic matter
Bfgj	Bf horizon further characterized by partial permanent or periodic reduction (indicated by grey colouration)
Bm	B horizon slightly altered by hydrolysis, oxidation and/or solution yielding a change in colour and/or structure

BSAF	Biota-to-soil accumulation factor
Bt	B horizon illuviated and enriched with silicate clay (usually occurring underneath an eluviated horizon)
C (horizon)	C horizon unaffected by pedogenic processes, and the accumulation of $CaCO_3$, $MgCO_3$ and more soluble salts
Cc	*Calamagrostis canadensis*
CCME	Canadian Council of Ministers of the Environment
CEC	Cation exchange capacity
Ck	C horizon characterized by the presence of carbonates
Ckgj	Ck horizon further characterized by partial permanent or periodic reduction (indicated by grey colouration)
CL	Confidence limits (set at 95%)
C:N	Carbon to nitrogen ratio
CWS	Canada-wide Standards
CV	Coefficient of variation
Dr	*Dendrodrilus rubidus*
Ea	*Eisenia andrei*
EC	Environment Canada
EC25 or EC50	Effective concentration (25 or 50% reduction relative to control response)
El	*Elymus lanceolatus*
ESSD	Estimated species sensitivity distribution
F1	Fraction 1 (consisting of hydrocarbons nC6 to C10)
F2	Fraction 2 (consisting of hydrocarbons >C10 to C16)
F3	Fraction 3 (consisting of hydrocarbons >C16 to C34)
F4	Fraction 4 (consisting of hydrocarbons >C34)
Fc	*Folsomia candida*
FH	F (fermentation) H (humus), encompassing an organic layer within soil referred to as the forest floor, situated above the mineral horizons
Fn	*Folsomia nivalis*
HPLC-FD	High Performance Liquid Chromatography coupled with Fluorescence Detection

IC25 or IC50	Inhibitory concentration (25 or 50% reduction in effect relative to control population)
ISO	International Organization for Standardization
LFH	L (leaf litter) F (fermentation) H (humus), encompassing an organic layer within soil referred to as the forest floor, situated above the mineral horizons
L	Loam (soil)
LAS	Linear alkyl benzenesulphonates
LC25 or LC50	Lethal concentration (25 or 50% lethality)
LS	Loamy sand (soil)
LSD	Least Significant Difference test (Fisher's)
MC	Moisture content
MTBE	Methyl tert butyl ether
NB	New Brunswick
OC	Organic carbon
OECD	Organization for Economic Cooperation and Development
Of/Oh	Organic (O) horizon characterized by the accumulation of fibric and humic materials
OM	Organic matter
On	*Oppia nitens*
ON	Ontario
P	Phosphorous
PAH	Polycyclic aromatic hydrocarbons
Pb	*Pinus banksiana*
Pg	*Picea glauca*
PHC	Petroleum hydrocarbon(s)
Pma	*Picea mariana*
Pmi	*Proisotoma minuta*
Pt	*Populus tremuloides*
SAR	Sodium Adsorption Ratio
Sc	*Solidago canadensis*
SCL	Sandy clay loam (soil)

SCWG	Soil Classification Working Group
SE	Standard error
SK	Saskatchewan
SL	Sandy loam (soil)
SRC	Saskatchewan Research Council
Tp	*Trifolium pratense*
US EPA	United States Environmental Protection Agency

1. INTRODUCTION

The overall goal of this research was to contribute to the development of soil toxicity test methods, using test species and soil indigenous to boreal regions, to support the derivation of realistic ecological risk assessments, as well as remediation objectives applicable to contaminated soils within these regions. As a result, the research presented in this thesis encompasses the development of a new soil toxicity test using oribatid mites, as well as other plants and soil invertebrates of significance to boreal regions.

The most abundant and diverse microarthropod present in boreal forest soils encompass oribatid mites (Crossley and Bohnsack, 1960; Walter, 1985; Heneghan et al., 1999) that significantly contribute to soil processes (e.g., nutrient cycling). However, despite their relevance to soil systems, and the demonstrative fact that they are good indicators of soil quality (e.g., Andrés, 1999; Behan-Pelletier, 1999), the number of ecotoxicity tests that incorporate oribatid mites in the assessment of contaminated soils are few relative to that of other soil invertebrates (e.g., lumbricids, enchytraeids, Collembola, isopods, gastropods). Therefore, the initial focus of this research entailed the development of a new soil toxicity test to include oribatid mites into a suite of soil tests (i.e., test battery) applicable to the assessment of contaminated boreal soils.

As such, the applicability of *Oppia nitens*, as a representative oribatid mite in forest soils was assessed by:

- determining which soil characteristics positively influence the reproductive success of *O. nitens*;
- comparing the importance of organic matter content on adult and immature stages;
- comparing the response between age-synchronized and unsynchronized test populations; and

- determining the applicability of using *O. nitens* in the assessment of reference soils amended with boric acid.

The research associated with the above objectives is presented in Chapter 3, the results of which were published in the journal of Environmental Toxicology and Chemistry.

Oribatid mites are sensitive to hydrocarbon pollution (Blakely et al., 2002). Therefore, further studies were designed to evaluate the toxicokinetics of a hydrocarbon pollutant (phenanthrene, as a model substance) in *O. nitens*. The research was designed to use a model substances and test soil, such that comparisons to other soil invertebrate species in the general literature was possible. The objectives of the research included:

- determining the toxicity of phenanthrene to adult and immature stages; and
- determining the bioaccumulation potential of phenanthrene to adult *O. nitens*.

The research associated with the above objectives is presented in full detail in Chapter 4.

Although the initial focus of research encompassed the development of a new oribatid mite toxicity test, a comparison of the sensitivity of *O. nitens* to that of other boreal species was warranted. As a result, a further study, using field-collected reference and contaminated soils, was completed using an expanded suite of boreal species: Collembola, earthworms and boreal tree and understory plants. The sensitivity of the boreal species was then compared to that of currently published standard soil toxicity test species (earthworms, Collembola and agronomic plants). Most forest soils have distinct horizon differentiation that can remain unperturbed despite the occurrence of contamination (e.g., as in the case of a surface spill); therefore, the test systems were modified to maintain certain horizons. Therefore, the study included the collection of individual soil horizons from the field, and used a layered horizon approach within the test vessels to mimic horizon depths observed in the field. This is in contrast to the traditional collection of soils, for example, from arable regions, where the soil is tilled, and horizons are disturbed resulting in a depth-based collection, and loss of horizon structure. As a result, the research objectives included:

- assessing the performance of boreal plant (6) and soil invertebrate (4) species using a standard formulated artificial soil, as well as two uncontaminated boreal forest soils;
- assessing the toxicity of a hydrocarbon and brine impacted site soil using the boreal and standard test species; and
- evaluating the use of a modified test system, whereby horizons were collected independently and reassembled within the test vessels; this is in contrast to traditional collection of soils, whereby they are collected by depth, irrespective of horizon.

The research associated with the above objectives is presented in Chapter 5, the results of which were published in the journal of Environmental Toxicology and Chemistry.

2. LITERATURE REVIEW

2.1. Introduction

Within the last two decades, soil ecotoxicology has taken on a central role in regulatory and provincial frameworks for the assessment of the effect and risk associated with soil contamination. Soils are a ubiquitous, but finite resource, ever contributing to the sustainability and quality of the biodiversity of terrestrial ecosystems ranging from minute microbial organisms to the support of our national productivity and quality of life. Soils are intimately entangled within the myriad of industrial activities (e.g., forestry, mining, oil and gas) that occur nation-wide, but are also vulnerable to contamination and subsequent deleterious effects, such as degradation, and loss of structure and ecological function, through the inadvertent release of pollutants. Within Canada, regulatory programs are recognizing the value of this finite resource, and the preservation of ecological integrity through the implementation of foe example , soil quality guidelines through the Canadian Council of Ministers of the Environment (CCME), Federal and Provincial contaminated sites programs, and priority assessments under major Federal programs (e.g., Chemicals Management Plan). Since 2004, Environment Canada (EC) has published standardized soil toxicity test methods (EC, 2004; EC, 2005a; EC, 2007) to support Federal and Provincial regulatory bodies in the assessment of contaminated soils, in addition to the assessment of the effectiveness of soil remediation technologies. This suite of methods complement existing international methods (e.g., ISO, 1993; ISO, 1998; ISO, 1990), but were developed in the context of field-contamination, adapting methodology for application to field soils reflective of Canadian ecosystems. However, the methods are limited in that the species and methodologies are adapted primarily to the evaluation of disturbed agronomic systems, which approximate to about 15% of Canada's landmass.

Boreal regions, on the other hand, account for over 50% of Canada's landmass, and resources within these regions significantly contribute to Canada's economy through forestry,

mining, and oil and gas activities. Although affected by pollution events, no standardized soil toxicity test methods exist nationally or internationally that specifically addresses these northern non-temperate ecosystems. Forest soils are unique from agronomic soils, in that they are not regularly cultivated, but exhibit well-defined succession of natural horizons of a forest floor (e.g., litter) and organic horizons, which cover diverse mineral soils (Fisher and Binkley, 2000). In 2003, Environment Canada hosted a workshop to define Canada's future needs for terrestrial toxicity testing. The results of this workshop prioritized the need for ecotoxicity test methods suited to boreal habitats, recognizing the importance of maintaining ecological relevancy and the provision of guidance in the sampling and handling of forested ecosystems (EC, 2003). In response, a long-term research program was initiated to develop a suite of plant, invertebrate and soil microbial tests applicable to Canadian boreal regions (e.g., forested and wetland habitats). The efforts described herein present the development (e.g., oribatid mite test) and application of a new suite of terrestrial test methods (plants, earthworms, Collembola and oribatid mites) applicable to boreal regions, using species and soils that naturally occur and/or were collected from within this regions.

2.2. Species Selection

The selection of test species requires a balance between ecological relevance representative of the structural and functional diversity present within a healthy soil community (e.g., across trophic levels), but also practical considerations for the functionality of the test (van Gestel, 1998; Stephenson, 2003). Therefore, a test battery approach, using multiple species and biological endpoints, is often employed in ecological assessments to better capture the structural and functional complexity of the soil system. This allows for the consideration of different routes of exposure, as well as varied toxicokinetics (e.g., biotransformation and detoxification), thus accounting for species and endpoint sensitivity.

Factors that influence the selection of species relevant to soil systems include:

- functional diversity (e.g., primary producers, herbivores, predators, fungivores, etc.) and taxonomical diversity,

- ecological relevance to geographical region of concern (i.e., occurrence and abundance),
- likelihood of coming into contact with contaminants (e.g., major portion of life cycle occurring within soil),
- stress tolerance, that is, insensitive to environmental factors, but exhibits varied sensitivity to multiple contaminants of concern (e.g., hydrocarbons, metals, pesticides, etc.),
- varying routes of exposure (e.g., dermal absorption, inhalation, ingestion and intestinal absorption, etc.),
- varying micro-habitats (e.g., soil pore water versus air-filled pore spaces), and
- amenable to life cycle tests such that different life cycles can be assessed (e.g., egg or juvenile production, adult survival) (Stephenson et al., 2003; Römbke et al., 2006).

However, these factors must also be balanced against practical considerations conducive to experimental (e.g., laboratory or field-based) toxicological assessments that yield information within a reasonable amount of time. For example, ecotoxicological assessments often form the basis of time-sensitive site-specific ecological risk assessments and remediation programs applicable to contaminated sites. Practical considerations include: species controversy (e.g., non-endangered or rare), availability (e.g., seed sources for plants) and culturability (e.g., soil invertebrates), available definitive taxonomy for field-derived species, life cycle durations (e.g., short versus long generation time), cost considerations (e.g., time and equipment requirements), and test repeatability, robustness and standardization (van Gestel, 1998).

Such factors have been taken into consideration for the selection of an appropriate test battery for the assessment of contaminated soils in boreal forested regions. These forest soils are uniquely characterized by their well-developed horizon structure, with thick forest floor and organic layers covering mineral soils. The low evapotranspiration rates and shortened growing season yield relatively cool moist conditions save for forests present on well-drained landforms or in regions with very dry summers (Fisher and Binkley, 2000). The conditions and soil types within these regions give rise to plant and soil invertebrate species that differ from those represented by current standard test methods, which are representative of agronomic soil regions (e.g., EC, 2004; EC, 2005a; EC 2007) within Canada.

Recently, efforts have been directed to the development and use of boreal and northern species relevant to the assessment of contaminated soils prevalent within these regions (EC, 2010). This initiative has led to the proposed use of boreal tree and understory plant species based on prevalence, stress tolerance to environmental factors, seed availability, germination and growth under standardized test conditions, and sensitivity to diverse contaminants (SRC, 2003). The species include coniferous (*Pinus banksiana*, *Picea glauca* and *Picea mariana*) and deciduous (*Populus tremuloides*) tree plants, as well as understory species (e.g., *Calamagrostis canadensis* and *Solidago canadensis*). Not only do these species dominate the boreal forest landscape (Fisher and Binkley, 2000), they are affected by industrial activities, and also used in reclamation efforts (e.g., *P. glauca*, *P. tremuloides*, and *P. banksiana*) (Khasa et al., 2005; Sorenson et al., 2011).

Similarly, the need for representative soil fauna is required, as this community of species work in tandem on processes such as decomposition of organic materials, and mineralization of nutrients, on which the flora also depend. Consequentially, the need for boreal forest invertebrate test species has been recognized (Römbke et al., 2006), with recommendations based on factors such as origin (i.e., native), frequency and abundance, habitat, stress tolerance (sensitive to pollutants but not environmental factors), but also practicality in acquiring and culturing species, in addition to definitive taxonomy. Based on a review of these criteria, and prevalence in European and Canadian boreal regions, recommendations for species of lumbricids, enchytraeids, Collembola, nematodes, carabids and gamasid (predatory mite) were put forth (Römbke et al., 2006). However, recommendations for Oribatida, Staphylinidae, Isopoda and Araneida were lacking, mainly because species, suitable for the assessment of boreal soils, could not be identified. Of the groupings identified, research efforts have focused on the use of lumbricids, Collembola and oribatid mites as a starting point for the development of soil test species applicable to boreal forest soils.

2.2.1. Plants

Phytotoxicity tests have been widely used in ecological assessments to assess soil contamination for a variety of contaminants (e.g., Kaputska, 1997; Saterbak et al., 1999; Stephenson et al., 2002; Fernandez et al., 2006). Many of these studies utilize national and international standard test protocols (OECD, 1984; USEPA, 1989; ISO, 1993; ISO, 1995; ASTM, 1999; EC, 2005a), which are limited in scope by the selection of garden and crop species. However, the use of native plant species for region-specific ecological assessments is recognized, and has so far been incorporated into the evaluation of the effects of pesticides (Boutin et al., 2004; Mihajlovich et al., 2004; Olszyk et al., 2008; Boutin et al., 2010) and metals (Smith et al., 2013; Thomas et al., 2014). Given the degree to which resources within the boreal ecozones are being developed (e.g., mining and oil sands), the need for the inclusion of boreal plant species as standard test species should become innate to ecological risk assessments and reclamation efforts within these regions. However, there are relatively few ecotoxicological studies evaluating for effects on boreal forest plants. Variations in salt tolerance, and the effects of salinity on boreal forest plants have been evaluated in support of reclamation efforts (Howat, 2000; Khasa et al., 2002; Lilles et al., 2012), with recent research on the effects of salinity associated with oil and gas development (Renault et al., 2000; Croser et al., 2001; Franklin et al., 2002; Khasa et al., 2004; Leskiw et al., 2012). The direct and indirect effects of heavy metals in boreal soils have long been studied (e.g., Hutchinson and Whitby, 1977; Feisthauer et al., 2006), with evidence of adaptive tolerance for some boreal species (e.g., red maple and white birch) (e.g., Watmough and Hutchinson, 1997; Kirkey et al., 2012). However, the need for integrated measures of effects of petroleum hydrocarbon (PHC) contamination in forest soils has been acknowledged, as such evaluations are generally rare (Robertson et al., 2007).

2.2.2. Earthworms

Earthworms present in boreal and northern landscapes within Canada have been a controversial point of discussion in recent years. Earthworm species native to northern areas of the North American continent (particularly Canada) were hypothesized as becoming extinct during the glaciation period (Gates, 1982). However, the recolonization of Canada by European

settlers also resulted in the introduction of lumbricid earthworm species, many of which have since invaded boreal forests as a result of human activity (Cameron et al., 1997; Addison, 2009; Moore et al., 2009). In many agricultural ecosystems, earthworms have been recognized for their positive ability to enhance soil structure and fertility (Clapperton et al., 1997). However, their ability to affect soil physical properties and nutrient cycling within the forest floor have also yielded changes to plant, microbial and other soil fauna populations (Edwards and Bohlen, 1996; McLean and Parkinson, 2000; Bohlen et al., 2004a; Bohlen et al., 2004b). For example, the burrowing activity of anecic earthworms (e.g., *Octolasion tyrtaeum*), have been shown to destroy soil habitats, through the mixing of litter into mineral horizons, removing food resources for soil arthropods, and affecting soil microbial biomass (Eisenhauer et al., 2007). On the other hand, an increase in microarthropods have also been observed in the middens of *Lumbricus terrestris*, also an anecic species (Mauran et a., 1999). Regardless, the impact of invasive earthworms on soil structure has the potential to change significant aspects of ecosystem function and biodiversity (Addison, 2009).

Given the potential for significant changes to the forest ecosystem, why include lumbricid species as new test species for the assessment of contaminated soils in these regions? The fact remains that these species are and will continue to be present in boreal forests, as a result of human activities and the economic value associated with resources present in explored and unexplored boreal regions. For example, the annual boreal forest marketable value across all products is estimated at $37.5 billion (Canadian) (Sanderson et al., 2012), with further estimates of nonmarket ecological goods and services (e.g., environmental or ecological services) at $703 billion (Anielski and Wilson, 2009). Therefore, characterizing the effect of contaminants on the soil processes, invertebrate and plant communities must also take into account the reality of these ecosystem changes. For regions that have been affected by human activity for decades (e.g., forestry, oil & gas development, mining), it would be unrealistic to assume that a site could be remediated to conditions inherent to that area prior to the disturbance. It is likely that remediation efforts, although best directed to maintain ecological function, will also induce change through attempts to reclaim sites to their inherent natural conditions. Earthworms migrate at a relatively slow rate of 5 to 10 m per year (Marinissen and van den Bosch, 1999), of which dispersal may increase as a result of human activity (e.g., dispersal through road

allowances, vehicle use, etc.) (Cameron et al., 2007; Moore et al., 2009). However, limitations of litter type, soil pH and cold tolerance may lessen or inhibit the rate at which earthworms can migrate north through boreal, and possibly taiga, ecosystems (Addison, 2009).

Of the earthworms present in boreal regions two species, *Dendrodrilus rubidus* and *Dendrobaena octaedra*, both of which are present in boreal forests (McLean and Parkinson, 1997; Dymond et al., 1997; Cameron et al., 2007), have been evaluated for incorporation into a test battery suited to the assessment of contaminated boreal soils (EC, 2010). Both are epigeic species suited to the colonization of undisturbed forest floor (Dymond et al., 1997), are acid-tolerant to soil conditions present in coniferous forests (Reynolds 1977; Tiunov et al., 2006); and display cold-tolerance up to -15°C (*Dd. rubidus*) and -20°C (*D. octaedra*) (Tiunov et al., 2006). However, research demonstrates that the sensitivity to certain contaminants (e.g., heavy metals) increase under cooler conditions (e.g., -6°C) as a result of affecting freeze tolerance mechanisms (Bindesbol et al., 2009a). Freeze tolerance occurs as a result of changes to lipid chemistry and from the accumulation and concentration of glucose as a cryoprotectant as bodily fluids begin to freeze (Overgaard et al., 2007; Holmstrup et al., 2007). Heavy metal (e.g., nickel, mercury and copper) toxicity for *D. octaedra* was greater at lower temperatures, as metals affect membrane lipid composition through lipid peroxidation (Bindesbol et al., 2005; Bindesbol et al., 2009b). In contrast, the sensitivity of *D. octaedra* to polycyclic aromatic hydrocarbons (PAH) (pyrene and phenanthrene) was less at cooler temperatures (e.g., -6°C versus 15°C), and although accumulation did not differ, the authors hypothesized that increased membrane fluidity brought about by the contaminant's lipophilicity conferred an advantage during freezing (Bindesbol et al., 2009a). Although relatively few toxicity data exist, *D. octaedra* tend to have greater sensitivity to metals relative to other earthworm species (Bindesol et al., 2009a); *Dd. rubidus* accumulates metals to varying extents relative to other earthworms (Morgan and Turner, 2005), but has relatively high metal (e.g., arsenic and copper) tolerance (Langdon et al., 2001; Arnold et al., 2008), although no direct comparative laboratory toxicity data could be found for both species. *Dendrobaena octaedra* demonstrates varied sensitivity to PAHs (e.g., pyrene and phenanthrene) (Bindesol et al., 2009a), salts (Chapter 5), and pesticides (e.g., abermectin, carbendazim, fenitrothion) (Addison and Holmes, 1995; Bindesol et al., 2009a).

2.2.3. Collembola

Collembola comprise a significant portion of forest soil fauna, and are important in the maintenance and preservation of soil structure through their contributions to decomposition and nutrient cycling (Seastedt, 1984). Their feeding habits mainly encompass the consumption of fungal hyphae and spores, bacteria, and decaying plant material (Hopkin, 1997); they also serve as prey for higher trophic levels (e.g., predatory mites). There exists a multitude of studies on the use of collembolan species as part of soil toxicity testing, ranging from single-species tests to soil microcosm tests. Standard soil test methodologies are available for many collembolan species (e.g., *Folsomia candida*, *Folsomia fimetaria*, *Orthonychiurus folsomi* (formerly *Onychiurus folsomi*) (ISO, 1999; EC, 2007; OECD, 2009), with recent efforts to develop new methodologies inclusive of species native to regions of concern (e.g., Greensdale and Vaughan, 2003; Santorufo et al., 2012). Two collembolan species were collected from forest soils as potential test species for Canadian boreal regions: *Folsomia nivalis*, and *Proisotoma minuta* .

Folsomia nivalis is a parthenogenetic species, with widespread nearctic distribution (Christiansen and Bellinger, 1998) and has been recommended for use in forest-related toxicological studies because of its prevalence (Parsons and Parkinson, 1986) and high reproduction potential (Addison, 1996). However, only one study reports the use of *F. nivalis* as a toxicity test species in the evaluation of contaminated forest soil (Addison, 1996: tebufenozide).

Proisotoma minuta, in contrast to *F. nivalis*, is a sexually-reproducing species (Massoud and Betsch-Pinot, 1974), with wide distribution within Canada (Dodd and Addison, 2010) and worldwide. As a soil toxicity test species, *P. minuta* has been used more frequently than *F. nivalis*, with documented observations of its life history traits when cultured under laboratory conditions (Park, 2007). The species exhibits a fairly wide tolerance to culture temperatures (16 to 23°C), with hatching rates optimized at 23°C (i.e., 6 ± 0.5 days) (Park, 2007); tolerance to soil pH has been suggested between 3.7 and 5.4 (Greensdale and Vaughan, 2003), although successful tests have been conducted at a soil pH between 6 and 7 (Dodd and Addison, 2010); one study using a field soil of pH 8.3 did not support *P. minuta* survival or reproduction, but

other contributing factors may have been present, as the study was conducted as a multiple-species soil microcosm study (Domene et al., 2010). The sensitivity of *P. minuta*, relative to other collembolan species, seems to vary with contaminant. For example, *P. minuta* was equally as sensitive as *Folsomia candida* to exposure to the organic contaminants, phenol, nonylphenol, methyl tert butyl ether (MTBE), as well as petroleum hydrocarbon-impacted soil (Greensdale and Vaugh, 2003; Dodd and Addison, 2010). *P. minuta* was less sensitive than *Onychiurus folsomi* to MTBE (Dodd and Addison, 2010). The toxicity and capacity of *P. minuta* for oxidative metabolism when exposed to insecticides (endosulfan, aldrin and dieldrin) have also been assessed, using soil media (Park and Lees, 2004; Park and Lees, 2006). Their sensitivity to metals vary, with reports of equivalent sensitivity to *F. candida* for cadmium and lead (Nursita et al., 2005), equal and greater sensitivity to copper (Greensdale and Vaughan, 2003; Nursita et al., 2005), greater sensitivity towards zinc (Nursita et al., 2005), and less sensitivity to arsenic (Greensdale and Vaughan, 2003). *Proisotoma minuta* also displays equivalent sensitivity to other collembolan species (e.g. *F. candida*, *F. nivalis*) when exposed to salts (Owojori et al., 2009).

2.2.4. Acari with special emphasis on Oribatida

The main objective of this thesis was the development of a new bioassay to encompass oribatid mites in a test battery suitable for the assessment of contaminated boreal soils, as these species are under-represented in general. Therefore, an extensive review of Acari, oribatid mites, and the use of specific oribatid mite species in toxicity tests is presented below.

Acari (or Acarina) (mites) are arthropods belonging to a subclass of Arachnida, and date as far back as the Devonian period (i.e., approximately 400 million years) (Norton et al., 1988). Common to other arachnids (e.g., spiders, scorpions), adult mites have four pairs of legs, chelicerae (modified mouth appendages used to pierce, tear, chew and suck), and pedipalps (antennae-like appendages near the mouth with various functions; e.g., manipulating food, sperm transfer, chemosensory), but lack primary segmentation (e.g., segments such as the cephalothorax and abdomen appear fused) and have hexapod larvae (however, each successive stage is characterized by four pairs of legs). Approximately 55,000 species have been described

(Walter and Procter, 1999), representing only about 5 to 10% of the species estimated to exist (Krantz and Walter, 1999). Acarines are ubiquitous, occupying a number of terrestrial, aquatic and arboreal habitats, as well as nests and other living organisms (e.g., birds); however, most free-living mites primarily or secondarily inhabit soil and litter (Krantz and Walter, 2009).

The Acari are divided into three major lineages: (i) Opilioacariformes comprising a single order and family (Opilioacarida, Opilioacaridae); (ii) Acariformes with over 300 families and 30,000 species; and (ii) and Parasitiformes comprising three orders (Ixodida, Holothyrida, and Mesostigmata) with over 70 families and 10,000 species, the majority of which belong to the Mesostigmata (Walter, 1996). The Acariformes can be divided into two major lineages: (i) Sarcoptiformes, which consist of Oribatida and Astigmata; and (ii) Trombidiformes, which consist of Prostigmata. The Acariformes are unique relative to other mites, in that these mites contain optically active chitin that is bifrigent under polarized light, and specialized setae (trichobothria) which are sensitive to vibrations and air currents (Proctor, 1998).

2.2.4.1. Oribatida

Oribatid (Oribatida = Cryptostigmata) mites are free-living and typically the most abundant and diverse microarthropod present within forest soils (Crossley and Bohnsack, 1960; Walter, 1985; Heneghan et al., 1999), with densities ranging from up to 200,000 (Maraun and Scheu, 2000) to approximately 500,000 individuals (ind) m^{-2} (Behan et al., 1978). Oribatid mites significantly contribute to nutrient (e.g., nitrogen) immobilization and mineralization (Singh et al., 1996; Johnston and Crossley, 2002; Hansen, 2000), and soil formation (Coleman et al., 2004). The direct contribution to nutrient cycling is a result of their saprophytic habits, consuming (grazing or shredding) organic debris (e.g., detritus), and fungus; endophagous species of oribatid mites also contribute to the breakdown of more recalcitrant substrates such as coniferous litter (e.g., needles) and woody debris (Seastedt, 1984). Oribatid mites fragment the organic matter, and through digestion, increase the surface area of the organic debris, facilitating further breakdown by microorganisms (Moore et al., 1988). In addition to their grazing habits, oribatid mites contribute to the translocation of bacteria and fungi through surface accumulation on their exoskeletons, and the distribution of their faecal pellets. Some oribatids are also

predaceous, mainly feeding on nematodes and microfauna, with some evidence for necrophagy (feeding on dead animals) (Behan-Pelletier and Eamer, 2004; Norton and Behan-Pelletier, 2006).

Oribatid mites are typically medium to dark brown, with some exceptions, and are on average 300 to 700 μm, but can range in size from 150 to 1500 μm (Behan-Pelletier, 1999). Growth and maturity is characterized by six post-embryonic developmental stages (an inactive prelarva, and an active larva, protonymph, deutonymph, tritonymph, and adult) whereby growth is accomplished with the shedding of exoskeleton. Some aspects of development are unique in that the legs of subsequent instars are formed within the body, rather than within the hull of previous instars legs (Proctor, 1998). The moult (ecdysis) involves a pre-ecdysial (development of integument) resting stage, characterized by immobility for a prolonged period of time, and depending on the species, can occupy up to one third of a mite's total life span (Luxton, 1981). The shedding of exoskeleton stops with the final developmental stage, as the exoskeleton within the adult form becomes hardened and sclerotized. Melanization is also typical, exhibited by the medium to dark brown colouration typical in most mites.

The life-history traits of oribatid mites are generally described as "*k*-selected", characterized by low metabolism, slow development, and low fecundity. However, given the abundance and diversity of oribatid species, they exhibit broad and opportunistic feeding habits (Norton, 1985), and are able to disperse (albeit slowly, and primarily as adults) and colonize different soil habitats and horizons, and occupy varied trophic levels (Behan-Pelletier, 1999). The life-span of oribatid mites (i.e., egg to adult) vary between species, ranging from a few weeks to several months to a year in temperate soils (Norton, 1985), but range from three to as long as seven years in cooler climates (Cannon and Block, 1988; Webb, 1989). However, time to maturity has mainly been documented under laboratory conditions, of which temperature can affect the maturation duration (Norton, 1994). In fact, some species have the ability for super-cooling during freezing temperatures, thus also possibly extending longevity, but also allowing for winter dormancy (Cannon and Block, 1988).

Oribatid mites exhibit iteroparity, that is, the ability to produce successive generations within one year. Some Brachypylina species also demonstrate aparity, whereby if the gravid

female dies before laying her last clutch of eggs, the eggs will nonetheless develop and larvae will hatch from within the dead carcass (Norton, 1994). Reproduction is such that fertilization is rarely direct (Norton, 1994), and involves the male depositing a free-standing spermatophore for the female; this process may or may not include courtship rituals or signals (e.g., a series of spermatophores) to encourage females to find the spermatophore for increased chances of fertilization. In addition to sexual reproduction, thelytoky, the parthenogenetic production of females from females, is also quite common among oribatid mites, thus only one individual is required to continue the population. However, other mites are arrhenotokous, whereby a female will produce a haploid male within the progeny to either mate again with the mother or other females; the resultant second generation will either by haploid (male) or diploid (female). It has been observed that net reproductive rates are such that population densities minimally vary from one year to the next (Norton, 1994), although fecundity has been estimated to be near or slightly above unity (i.e., at least one surviving progeny per adult) (e.g., Mitchell, 1977).

The combination of a long life-span, coupled with iteroparity, is thought to buffer population losses during stressful conditions (e.g., scarcity in resources, disturbances) (Hansen, 2000). Mortality losses in nature are usually observed in immatures (Norton, 1994; Behan-Pelletier, 1999), most likely because of predation; immatures would be highly vulnerable during their resting stage in between moults. Immature forms are also not fully sclerotized (relative to the adult form), and are therefore more susceptible to changes in environmental conditions (e.g., humidity), as well as predation. However, although immature mortality can be quite high (e.g., up to 90% in some instances (Norton, 1994)), the presence of immature forms can contribute to one third of the total oribatid mite population within soil, accounting for 50 to 70% of the overall observed metabolism within the oribatid mite community (as cited in Seniczak, 1975a). Adult mortality may also result from predation by larger arthropods, such as beetles or ants.

The long time to maturity also corresponds to the need for a longer life-span to accumulate sufficient resources and energy for reproduction. As a result, adult forms have developed effective defense mechanisms through protective setae, camouflage (e.g., pigmentation), waxy exudates, defensive glands, cuticular hardening and protective structures (e.g., tectum that protect soft cuticular regions) (Norton and Behan-Pelletier, 2006). The

cuticular hardening is most likely derived from mineralization, rather than sclerotization (Norton and Behan-Pelletier, 2006), as oribatid mites can sequester calcium and other minerals (Norton and Behan-Pelletier, 1991; Lebrun and van Straalen, 1995; van Straalen et al., 2001) within their cuticle. Some mites can also incorporate organic and inorganic debris within and on their cerotegument (i.e., on top of their cuticle) (e.g., Damaeidae) (Norton and Behan-Pelletier, 2006).

2.2.4.2. Oribatid mites in soil toxicity testing

The use of oribatid mites as bioindicators of environmental disturbances have been extensively reviewed (Lebrun and van Straalen, 1995; Behan-Pelletier, 1999; Gergócs and Hufnagel, 2009). Accordingly, oribatid mites have been used in the assessment of soil contamination, as indicators of soil quality (Al-Assiuty et al., 2000), with demonstrated susceptibility to metal (Denneman and van Straalen, 1991; Siepel, 1995; Skubała and Kafel, 2004; Skubała and Zaleski, 2012), pesticide (Prinzing et al., 2002; Beck et al., 2004; Adamski et al., 2007) and hydrocarbon pollution (Blakely et al., 2002), including sewage sludge and biosolid application to soils (Andrés, 1999; Minor and Norton, 2004; Andrés et al., 2011). Several studies also demonstrate an increased sensitivity of oribatid mites to environmental disturbances relative to other Acari and soil arthropods (e.g., Bengtsson and Rundgren, 1988; Battigelli et al., 2004; Minor and Norton, 2004). Their life-history characteristics (e.g., low metabolism, slow development, low fecundity) and slow dispersal capabilities limit their ability to adapt to short-term disturbances, leading to a rapid decline in population, which can in turn, be detected as a sign of environmental degradation (Lebrun and van Straalen, 1995). In contrast, certain species, demonstrating *r*-selected strategies (e.g., short life-cycle, higher reproduction, fast colonizers) can also be used as bioindicators. These 'generalist' species, such as those found in the families Brachychthoniidae, Tectocepheidae and Oppiidae, are often thelytokous and abundant in disturbed habitats (Behan-Pelletier, 1999). Further evidence suggests that an evaluation of the percent distribution of Oribatida, based on their life-history traits, can be used to monitor ecosystem quality over time (Siepel, 1995; Siepel, 1996).

The specific use of oribatid mites in soil ecotoxicological testing has also been reviewed (Lebrun and van Straalen, 1995), which led to the subsequent development of a lethality and

reproduction laboratory soil assay using the parthenogenetic species *Platynothrus peltifer* (C.L. Koch, 1839) (van Gestel and Doornekamp, 1998). The test methods included both dietary and soil exposure studies, whereby the soil exposure studies demonstrated greater sensitivity to selected toxicants (copper and sodium salt of linear alkyl benzenesulphonates (LAS)) (van Gestel and Doornekamp, 1998). The results of this research demonstrated the necessity of a soil exposure system to account for multiple exposure pathways. However, standardization of a test method using *P. peltifer* was limited by the need to use field-collected specimens because of difficulties in the establishment and maintenance of laboratory cultures. Although the tests were effective at discerning effects on reproduction after exposure to contaminated soil, the tests were compromised by high adult mortality, and were lengthy (e.g., 6 to 12 weeks for reproduction endpoint) to accommodate the species' long development cycle (e.g., > 150 d to maturity) (van Gestel and Doornekamp, 1998). Although prevalent in boreal and arctic ecosystems, difficulties associated with culturing and testing also led to the recommendation for exclusion of this species from soil toxicity test method development (Römbke et al., 2006).

Additional studies have incorporated the use of *Archegozetes longisetosus* (Aoki, 1965) as a laboratory test species (Seniczak and Seniczak, 2002; Köhler et al., 2005; Seniczak, 2006; Seniczak et al., 2006; Heethoff et al., 2007; Seniczak et al., 2009) as this species is also parthenogenetic, easily cultured under laboratory conditions, and characterized by a short generation time with relatively high fecundity (Heethoff et al., 2007). However, this species is limited to a pan-tropical distribution; therefore, the relevance of this species to non-tropical habitats is questionable, particularly when assessing soils of boreal and northern regions. Of other mite species, much research has been conducted to standardize *Hypoaspis* (*Geolaelaps*) *aculeifer* as a standard test species (Smit et al., 2012; OECD, 2008); however, this species occupies a higher trophic level through its predatory habits, and does not represent the ecological niche that oribatid mites occupy.

Of oribatids, many studies have incorporated the use of *P. peltifer* in laboratory and field settings, as the use of this species dominates the paucity of toxicological studies available on mites in general. Laboratory studies demonstrate the sensitivity of *P. peltifer* to metals, with increased sensitivity to cadmium and lead (van Straalen et al., 1989; Khalil et al., 2009) and

moderate sensitivity to copper (Denneman and van Straalen, 1991). Comparison sublethal studies with other soil invertebrates have shown *P. peltifer* to be more sensitive to dietary cadmium than the Collembola *Orchesella cincta* and *Folsomia candida* (van Straalen et al., 1989; Crommentuijn et al., 1995); a comparison of lethality demonstrated the following degree of sensitivity: *O. cincta* > *P. peltifer* > the isopod *Porcellio scaber* > *F. candida* (Crommentuijn et al., 1995). However, a comparison of lethal body concentrations of dietary cadmium demonstrated that *P. peltifer* was less sensitive than the Collembola *O. cincta* and *Tomocerus minor*, but more sensitive than the isopods *P. scaber* and *Oniscus asellus* (Crommentuijn et al., 1994). Oribatid mites are known to accumulate metals, but accumulation is species-specific (Siepel, 1995; Zaitsev and van Straalen, 2001; Skubała and Kafel, 2004; Skubała and Zaleski, 2012). Some authors have demonstrated an increased susceptibility to heavy metals relative to other arthropods (e.g., Bengtsson and Rundgren, 1988; Hågvar and Abrahamsen, 1990), which may be in part be due to their saprophagous feeding habits, as fungi have been shown to efficiently accumulate heavy-metals (Khan et al., 2000). Accumulation of heavy metals may also occur through the simultaneous sequestration of calcium for mineralization of the cuticle or other essential minerals required for metabolic processes (Roth, 1993; Janssen and Hogervorst, 1993). In fact, low doses of certain metals (e.g., copper and lead) have stimulatory effects on reproduction at the individual level (Denneman and van Straalen, 1991), but also at the population level with regards to species richness and abundance (Seniczak et al., 1997). Oribatid mites also have the innate ability to compartmentalize metals, which allows for them to buffer against population losses in contaminated soils (Khalil et al., 2009). Aside from storage in the cuticle, Ludwig et al. (1992) demonstrated the accumulation of lead in schlerites present within proventricular glands within the oribatid mite, *Chamobates borealis*. The spherites function in pH and calcium regulation, and therefore, the authors suggested a detoxification mechanism through storage and immobilization of the metal through the uptake of calcium salts, which raised the pH within these glands. Further evidence also suggests the extrusion of the spherites into the lumen of the alimentary canal (as cited in Seniczak and Seniczak, 2002).

Although research on metals dominates toxicological studies associated with oribatids, researchers have also evaluated the effect of pesticides in laboratory and field-settings. In general, pesticide toxicity is substance and species-specific (e.g., Al-Assiuty and Khalil, 1995;

Cortet et al., 2002; Prinzing et al., 2002), with some species negatively affected (e.g., azadirachtin (Stark, 1992); 2,4,6-trinitrotoluene and p-nitrophenol (Parmelee et al., 1993), and others remaining unperturbed [e.g., chlorpyrifos (Stark, 1992; Michereff-Filho et al., 2004); endosulfan (Osler et al., 2001); zinc-manganese ethylene-bis-dithiocarbamate (Adamski et al., 2007)]. The use of biological control agents, such as those containing the bacterium *Bacillus thuringiensis* subsp. *kurstaki* have also failed to yield effects on oribatid mites (Addison et al., 2006; Oliviera et al., 2007).

With regards to hydrocarbon contamination, oribatid mites demonstrate some degree of sensitivity associated with smaller-ring polycyclic aromatic hydrocarbons (PAHs), with no effect associated with five-ring PAH compounds, such as benzo[a]pyrene and creosote (Blakely et al., 2002; Owojori and Siciliano, 2012). Similar results were found when *H. aculeifer* were exposed to benzo[a]pyrene for three weeks, in that no effects on adult survival or reproduction were observed at concentrations up to their highest test concentration of 947 mg kg^{-1} dry soil (Sverdrup et al., 2007). However, other researchers have found PAH contamination associated with a decline in the abundance of Acari in soil (Erstfeld and Snow-Ashbrook, 1999).

2.2.4.3. *Oppia nitens* (C.L. Koch)

Oppia nitens (C.L. Koch, 1836) is a Brachypylina species belonging to the family Oppiidae, the largest oribatid family with approximately 1000 species in 129 genera distributed across North America, the Palearctic and Holarctic regions (Ohkubo, 2001; Subías LS 2009: http://www.ucm.es/info/zoo/Artropodos/Catalogo.pdf). This species is typically found in the upper organic soil horizons, rich in mycelium and decayed organic matter, although some have been document in forest trees, with some smaller species (e.g., *O. minus*) being found in deeper mineral horizons (Seniczak, 1975a). Its feeding activities contribute to carbon mineralization and nitrogen and phosphorous release through the deposition of faecal pellets. *Oppia nitens* has been documented as a polyphagous fungivore, but does show some selective feeding preferences (Seniczak and Stefaniak, 1978). Singh et al. (1996) observed a strong preference for ground leaf litter mixed with dried mushrooms, with a moderate preference for leaf litter or mushrooms alone, and very little preference for granulated yeast; however, *O. nitens* has been successfully

reared on granulated yeast for at least two years prior to the research presented in this thesis. Other feeding substrates in nature may include lichens, raw humus and carrion (dead and rotting tissue), and the species may be predatory (Seniczak, 1975b; Seniczak and Stefaniak, 1978); in fact, under laboratory conditions, cannibalism was observed when the mites were given *Trichothecium roseum*, of which this food substrate was not preferred (Stefaniak and Seniczak, 1981). It has also been observed that the immature forms contained a more abundant and active microflora than adult specimens when gut contents were observed (Seniczak and Stefaniak, 1978), and therefore, this provides another instance where immature forms may have a significant impact on decomposition relative to adults. Of note for laboratory-reared populations, Stefaniak and Seniczak (1981) observed that a monotonous diet resulted in decreased alimentary microflora, mite activity and development of consecutive generations.

There is conflicting information regarding reproductive mode (i.e., sexual or parthenogenetic); however, observations of spermatophores under laboratory conditions (Stefaniak and Seniczak, 1981) are suggestive of sexual reproduction. There is no documented sexual dimorphism exhibited within the species (i.e., males do not differ in size from females), and sexual dimorphism has not been observed to date. *Oppia nitens* eggs are oval, whitish with a smooth surface, with sizes ranging from 90 to 150 μm (Seniczak, 1975b); personal observation (J. Princz) seems to indicate that eggs hatch within about one week of oviposition. Immature forms are also whitish in colouration, with larvae approximating 200 μm in length by 105 μm in width, and tritonymphs approximating 372 μm in length by 195 μm in width; a full morphological characterization is provided in Seniczak (1975b). Newly emerged adults are a semi-translucent pale golden-brown that darkens with melanization and sclerotization to a rich dark-brown within one week. Personal observations have found that the species matures within four weeks of hatching at 20 to 23°C. Sengbusch (1970) observed a developmental period of 45 to 46 days at 20°C, with females laying eggs only three months after hatching. Stefaniak and Seniczak (1981) observed a developmental period of 28 days at 23°C; and Yu et al. (1997) observed a developmental period of 2-3 weeks at 23°C with a fairly high humidity of 85 ± 5%.

2.2.4.3.1. *Oppia nitens* as a toxicity test species

Prior to the onset of this research, only one other study had used *O. nitens* as a test species. The toxicity of *Bacillus thuringiensis* toxins in transgenic cotton and potato was assessed (Yu et al., 1997) whereby ten adults were exposed to contaminated leaf discs or milled leaves in 0.7 g of autoclaved soil. The effect of dietary exposure only was evaluated on adult survival and reproduction, and no adverse effects were detected. These authors used cadmium as a positive control, but did not report the effects of the metal on either adult survival or reproduction. Since then, the applicability of using *O. nitens* survival and reproduction endpoints in the assessment of boreal forest soils has been demonstrated (Chapter 3). The products of this research demonstrated the insensitivity of adult survival to a diverse number of organic and mineral soils, but also demonstrated the limitations in using reproduction as a test endpoint. Juvenile production was limited by the degree of organic matter present within soil, and thus lower reproduction occurred in mineral soils, relative to organic soils. These findings are not unexpected in that the oribatid mite community structure is predominantly driven by humus form (Maraun and Scheu, 2000). However, reproduction varied across different organic forest soil horizons, and it is uncertain what the effect of humus type might have on reproduction, as this has not been specifically evaluated to date. *Oppia nitens* also appear tolerable to varied soil pH, such that Owojori and Siciliano (2012) noted good reproduction in low pH soils (e.g., pH < 6.1), but a significant reduction in reproduction occurred at a soil pH ≥ 7.3. In general, the effect of soil pH seems to be species-specific, with some exhibiting narrow tolerance ranges, and others having relatively wider tolerances (Hågvar and Abrahamsen, 1980; van Straalen and Verhoef, 1997).

Additional research has yielded the use of *O. nitens* in behavioural testing (Owojori et al., 2011). The principle behind this testing relies on the ability of a soil organism to detect and respond (e.g., avoid) to either chemical or physical (e.g., soil texture) stimulus when presented with a choice of matrices (e.g., contaminated versus reference soil). Typically, these tests are shorter in duration (e.g., 24 to 72 h), can be used as screening studies for contaminated site assessments (Luz et al., 2004), and can be indicative of sublethal stress (e.g., toxicity) conditions (e.g., Yeardley et al., 1996; Luz et al., 2004). The study conducted by Owojori et al. (2011)

demonstrated that 24 h was sufficient for an *O. nitens* avoidance study, and that selected soil parameters (organic matter content, soil pH, clay content, and moisture) had little effect on avoidance behaviour. An assessment of metals and organics in soil revealed a median effective concentration (EC50) lower or similar to those affecting *O. nitens* reproduction for copper, zinc, geraniol and phenanthrene, but higher for cadmium, lead and boric acid (Owojori et al., 2011). Relative to other soil invertebrate species, *O. nitens* is as sensitive upon exposure to cadmium and lead, but less sensitive to copper and zinc (Owojori and Siciliano, 2012). In addition, *O. nitens* has been shown to accumulate metals (Owojori and Siciliano, 2012), and thus lends itself as an additional soil test species for the evaluation of bioaccumulation kinetics in metal contaminated soils.

2.3. Boreal Forest Soil Types of Significance

Canadian boreal forests represent the largest biome within Canada, accounting for 35% of Canada's land mass, and representing 30% of the world's boreal forests (http:/www.atlas.nrcan.gc.ca/site/english/learningresources/theme_modules/borealforest/index.html). The soils within these regions encompass well-defined horizon structure, typified by a thick forest floor and organic layers covering mineral soils. The forest floor and organic layers support a multitude of flora and fauna that in turn, contribute to the soil's structure and function through decomposition and mineralization processes (Letang and de Groot, 2012).

The development of standard test methods applicable to the boreal forest requires not only representative species, but also soils typical to the environment under study. As a result, five soil orders were selected to represent the variety of boreal forest soils: Podzolic, Luvisolic, Brunisolic, Chernozem and Gleysolic soils (Fig. 2.1). Boreal forest soils are generally dominated by Podzolic, Luvisolic, Brunisolic soils, with Organic soils (e.g., peat bogs) present across wetlands and moisture-rich (e.g., low-lying) areas. The northern edges of the boreal forest are dominated by Cryosols, recognized by the presence of permafrost. Cryosols were not evaluated as a soil collection event was not available at the time of this research, and different handling and testing methodology would have been required to maintain the permafrost

conditions. It is also likely that new species, other than those collected, would have been required for the evaluation of these unique soils.

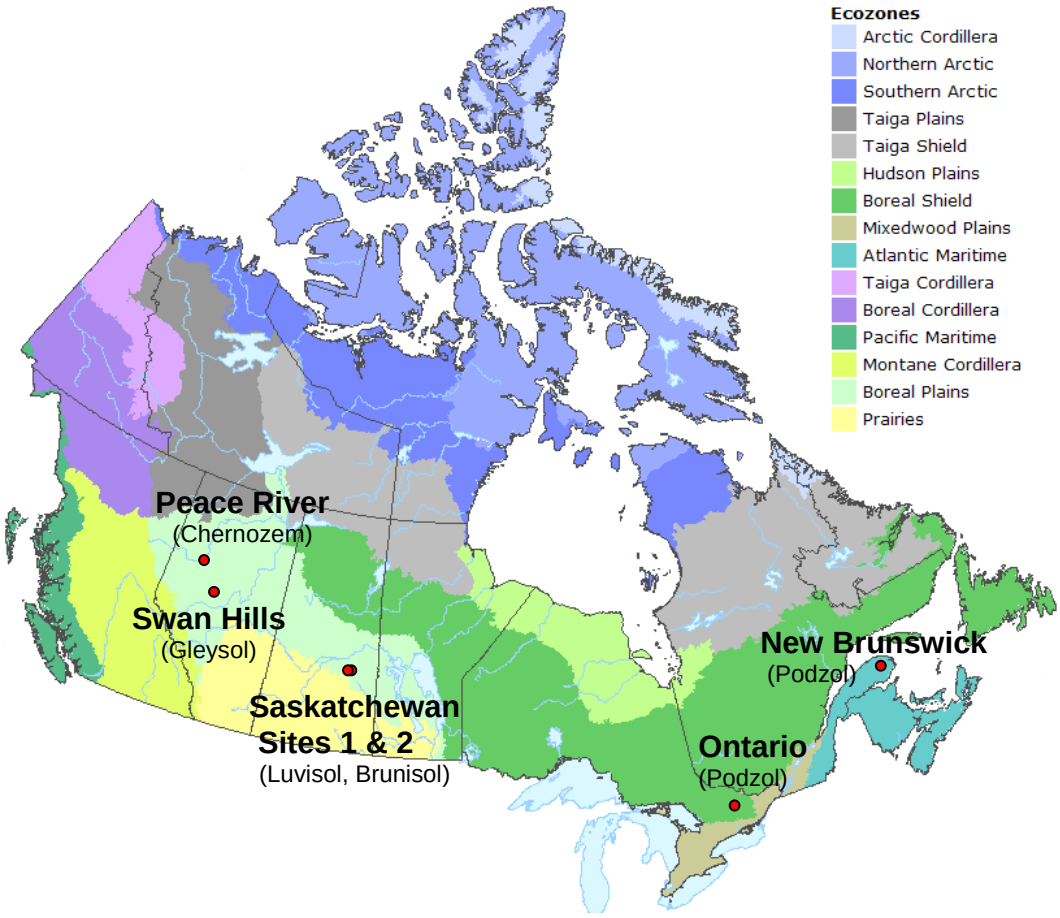

Fig. 2.1. The terrestrial ecozones of Canada, with boreal forest soil sampling locations and types indicated by dots (adapted from EC, 2010).

2.3.1. Podzolic soils

Podzolic soils are primarily found in temperate and boreal forest regions, with cool temperature and humid moisture conditions (Sanborn et al., 2011). The soils are typically acidic (e.g., pH 3.5 - 4.5) and characterized by a strongly leached or eluviated A horizon (Ae), with an accumulation of humidified organic matter and aluminium and iron deposits in the B horizon (Soil Classification Working Group, 1998). This soil order is the second most dominant order, occupying approximately 14% of Canada's land mass, followed by Cryosolic soils (Sanborn et al., 2011).

2.3.2. Brunisolic and Luvisolic soils

Brunisolic soils cover approximately 1.2 million km^2 (approximately 10%) of Canada's land mass (Smith et al., 2011), and is classified as an immature (or 'young') soil (i.e., weathered with moderate development, but less so relative to other soil orders), with a developed B horizon characterized by little accumulation of clay or amorphous material (Soil Classification Working Group, 1998). Brunisols are primarily forest soils, and tend to occur alongside Luvisolic soils (Smith et al., 2011).

In contrast, Luvisols are well-developed, with strong eluviation of clay deposits, and a higher soil pH (more so with depth) due to calcareous parent material (Soil Classification Working Group, 1998). Luvisols occupy approximately 645, 200 km^2 (approximately 9%) of land, and are typical to forested areas with a sub-humid to humid moisture regime (Lavkulich and Arocena, 2011). The accumulation of clay deposits through the B horizon form the basis of the Bt horizon, which is diagnostic of this soil order.

2.3.3. Chernozem soils

Chernozem soils occupy 4% of Canada's soils (Soil Classification Working Group, 1998), and are characterized by a brown to black A horizon that is rich in organic matter with little eluviation. This soil type dominates the Canadian prairies, and is typical to grassland ecosystems (Pennock et al., 2011). However, the soil collected for these studies were from the southern edge of the boreal forest, likely a transition zone between Canadian grasslands and boreal forest.

2.3.4. Gleysolic soils

Gleysolic soils occupy just over 1% of Canada (Soil Classification Working Group, 1998) and occur sporadically throughout the boreal forest. This soil type is typical to areas saturated with water and anoxic, resulting in the reduction of oxidized component (e.g., nitrates,

ferric oxide), yielding the gleyed colouration characteristic of these soils (e.g., grayish-green or grayish-blue) (Soil Classification Working Group, 1998; Bedard-Haughn, 2011).

2.4. Boreal Forest Contaminants of Concern

The boreal regions provide a multitude of natural resources that significantly contribute to Canada's economy via energy (e.g., oil and gas), mining and forest industries. Although of economic significance, these industries also inadvertently release pollutants that may pose a risk to the terrestrial soil ecosystem. For example, petroleum hydrocarbon contamination events, resulting from oil exploration, extraction, transport and processing, can result in surface and sub-surface soil contamination. In addition, associated activities (e.g., oil extraction) may also potentially result in excess brine or salt contamination. These materials affect the soil ecosystem by directly eliciting a toxic effect and indirectly through the alteration of the soil habitat (e.g., increased osmotic potential, increased soil hydrophobicity, altered food-web dynamics) (Alexander, 2000; Blakely et al., 2002; Khasa et al., 2002).

2.4.1. Petroleum hydrocarbon contamination

Approximately 60% of contaminated sites across Canada are impacted by petroleum hydrocarbon pollution, representing >250,000 sites (CCME, 2008). Petroleum hydrocarbons are a complex mixture of aliphatic and aromatic hydrocarbons, often containing varying amounts of nitrogen, sulphur and oxygen (Sugiura et al., 1997). Given the complexity of mixtures, the degree of contamination and subsequent toxicity varies depending on the type and source of the petroleum hydrocarbons, the site characteristics (e.g., soil type), and the time from the initial contamination event. Once introduced into the soil, petroleum hydrocarbons are subjected to biotic (e.g., microbial transformation) and abiotic processes (e.g., sequestration) that affect its composition and/or bioavailability. Subsequent effects on the soil ecosystem occur directly, as a result of the toxicity of chemical product(s) present, or indirectly, through the alteration of the soil habitat (e.g., increased soil hydrophobicity, decreased hydraulic conductivity and water retention capacity, altered food-web dynamics and decomposition processes) (Alexander, 2000; Blakely et al., 2002).

Within Canada, direction on the management of petroleum hydrocarbon-impacted sites is provided to industry and government regulators through the Canada-wide Standards for Petroleum Hydrocarbons in Soil, based on equivalent carbon ranges derived from boiling point ranges: Fraction 1 (F1), comprising hydrocarbons ranging from nC6 to C10), Fraction 2 (F2) (>C10 to C16), Fraction 3 (F3) (>C16 to C34) and Fraction 4 (F4) (>C34) (CCME, 2008). The standards outline national clean-up targets for each fraction, as well as for different land uses (e.g., agricultural, residential, commercial or parkland), based on fine and coarse-grained soils. These standards were derived based on two agronomic soils, using species recommended by standardized soil toxicity test methods (EC, 2004; EC, 2005a; EC, 2007), and fresh product (crude oil) that had been fractionated accordingly. Although the applicability of these standards to weathered contaminated sites is the subject of some debate in terms of level of conservation (Angell et al., 2012), there are provisions in some Canadian provinces for the use of site-specific soil ecotoxicological data in the derivation of remedial objectives, albeit using agronomic-based species. The debate over whether these standards are overly conservative relates to the behaviour of petroleum hydrocarbons in soil over time. Lower molecular weight hydrocarbons are more toxic than higher molecular weight constituents (Salanitro et al., 1997; Dorn et al., 1998; Cermak et al., 2010); however, lower molecular weight constituents (e.g., F1 and F2) are also more mobile, biodegradable and volatile, and thus may pose an acute risk to the soil ecosystem. As the contaminant ages within the soil, sorption-related processes, such as sequestration, hinder the biological uptake, assimilation and/or mineralization of the chemicals, making them more recalcitrant and decreasing their bioavailability (Kelsey and Alexander, 1997; Kelsey et al., 1997; White et al., 1997; Tang et al., 1998; White et al., 1999), and toxicity over time (Dorn et al., 1998; Robertson and Alexander, 1998).

Soil characteristics also influence the bioavailability and toxicity of petroleum hydrocarbons, with decreased availability and mineralization observed in fine-textured soils (e.g., clay, silt) relative to coarse-textured (e.g., sand) soils (Carmichael and Pfaender, 1997; Billeret et al., 2000; Carmo et al., 2000; Mulder et al., 2000; Amellal et al., 2001), as a result of greater surface area to mass ratio (Belfroid et al., 1996; Carmo et al., 2000). However, sorption over different particle size classes is thought to be partially controlled by the organic matter content (Belfroid et al., 1996), with finer fractions, as a result of their larger surface area,

containing more sorbed organic matter (Carmo et al., 2000), thus increasing the potential for sorption and partitioning (Kan et al., 1994; Amellal et al., 2001). Soil organic matter has a high affinity for nonpolar hydrophobic organic molecules, including petroleum hydrocarbons (Brusseau et al., 1991; Alexander, 1995; Dorn et al., 1998; Braida et al., 2001), and therefore the toxicity of such compounds is less in soils with high humus content (Salminen and Haimi, 1997). The sorption to soil particles increases not only the hydrophobicity of the soil, thus preventing water retention, but also increases the bulk density, limiting oxygen and nutrient transportation, affecting decomposition processes (Blakely et al., 2002). Such alterations to the soil habitat are of significance as boreal forest soils are characterized by thick organic layers that support diverse flora and fauna that contribute to the overall soil's structure and function. As a result, realistic measures of hydrocarbon toxicity in boreal regions should encompass the use of indigenous (or similar surrogates) boreal plants and soil organisms, that are applicable to the assessment of contaminated soils within these regions. Given the horizon differentiation characteristic of forest soils, environmental risk assessments, including site-specific remediation objectives, should also encompass the evaluation of organic soils for cases involving surface contamination.

The toxicity of petroleum hydrocarbons is dependent on petroleum source, soil type, and varies with species and endpoints (Salanitro et al., 1997), making direct comparisons across studies difficult. Species and end-point sensitivity varies with some observations of greater sensitivity for soil invertebrate sublethal endpoints (e.g., reproduction), relative to plants (Dorn et al., 1998; Cermak et al., 2010), with a recent study demonstrating comparable sensitivities (Angell et al., 2012). In general, petroleum hydrocarbons elicit toxicity via non-polar narcosis, yielding non-specific disruption of the cell membranes (Jager et al., 2000; Sverdrup et al., 2001). In addition to lipid solubilization as a result of direct contact with PHCs, plants can be indirectly affected at various stages of germination and growth. Decreased seed germination can occur as a result of reduced imbibition because of soil hydrophobic conditions (e.g., reduced water retention), but also through the inhibition of enzyme activity (e.g., amylase and starch phosphorylase) (Achuba, 2006). Plant growth can be inhibited by decreased oxygen, water and nutrient transport, either directly through lipid solubilization, or indirectly as a result of soil anaerobic or hydrophobic conditions (Rentz et al., 2003). Components of petroleum hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs) are also known to affect

physiological processes such as photosynthesis and mineral uptake (Sverdrup et al., 2003; Alkio et al., 2005). Specific mechanisms of toxicity for PAHs in organisms have been observed that involve biochemical activation, oxidative stress and the creation of carcinogenic reactive electrophilic species or biochemically reactive metabolites; some metabolites may not be toxic, but are hydrophilized for excretion. However, some researchers suggest that the P450 system and subsequent metabolism of PAHs is less active in invertebrates than vertebrates (Jones et al., 2008), with reports of no change in enzyme activity nor the presence of metabolites in earthworms exposed to pyrene (Achazi et al., 1998; Brown et al., 2004; Jones et al., 2008). In contrast, Stroomber et al. (2004) demonstrated that the earthworm *Eisenia andrei* could metabolize pyrene, but with much less efficiency than the Collembola, *Folsomia candida*, and the isopod, *Porcellio scaber*, and hence accumulated the PAH to a much higher degree. There are no studies that have evaluated P450-mediated detoxification of PAHs in oribatid mites, although P450 activity associated with the detoxification and resistance to acaricides has been demonstrated in other acarine species (e.g., Crampton et al., 1999; Pottleberge et al., 2008; Tirello et al., 2012).

2.4.2. Brine contamination

Activities associated with the oil and gas sector also have the potential to release saline produced waters (e.g., brine) through leaks in pipelines, oil wells or other infrastructure (Leskiw et al., 2012), resulting in soil salinization. Salinity is typically expressed or measured as either electrical conductivity or using the sodium adsorption ratio (SAR), which describes the relationship of sodium ions relative to the ratio of calcium and magnesium ions. Salt ions of relevance include Na^+, Cl^- and SO_4^-. Excessive salinization alters the soils physical and chemical properties affecting not only soil structure (e.g., porosity, density), but function through the disruption of osmoregulation (e.g., dehydration and concentration of ions) within the flora and fauna. Interestingly enough, the forest floor is thought to buffer against the effects of salinity for some plants, as the LFH layer contributes to salt removal through absorption, minimizing run-off, lowering surface evaporation rates, and enhancing leaching from the root zone (Leskiw et al., 2012); however, it is also within the LFH and organic horizons that soil organisms (e.g., micro- and macroinvertebrates) are most prevalent.

Sodic soils naturally exist across the boreal landscape, but are relatively rare and exhibit lower commercial productivity relative to non-saline soils (Lilles et al., 2012). In general, plant growth becomes affected when electrical conductivity exceeds 4 dS m^{-1} (Howat, 2000; Lilles et al., 2012). The effect of soil salinity on plant growth varies widely among species with salt-tolerant species capable of compartmentalizing (e.g., within vacuoles) and excluding or secreting salts through various processes, including leaf abscission (Howat, 2000). Sensitivity to excessive salts also varies within the plant's particular growth phase. Effects on seed germination and seedling emergence are dependent upon the seed's ability to overcome external osmotic potential for imbibition and subsequent embryo expansion (Al-niemi et al., 1992); differences in sensitivity relative to seed size have also been observed such that larger seeds are able to overcome osmotic stress given their greater solute content (Croser et al., 2001). Effects on plant growth are either indirectly affected by saline conditions via dehydration or directly through ion toxicity, which alters physiological processes such as enzyme activation, cell division and differentiation (Ayers et al., 1952; Al-niemi et al., 1992; Begum et al., 1992). Soil invertebrates are also equally susceptible to salt contamination through the disruption of the osmotic balance of bodily fluids (e.g., loss of body water, resulting in greater ionic concentration of salts), leading to documented effects on survival, growth and reproduction (Witteveen et al., 1987; Fischer and Molnár, 1997).

3. ORIBATID MITES IN SOIL TOXICITY TESTING: THE USE OF *OPPIA NITENS* (C.L. KOCH) AS A NEW TEST SPECIES[1]

3.1. Preface

Given their significance abundance, and contribution to soil nutrient cycling, oribatid mites remain under-represented in soil ecotoxicological testing. As a result, this chapter introduces the use and applicability of *Oppia nitens*, a representative oribatid mite, as a test species for use in boreal forest soils. The study evaluated the use of two toxicological endpoints (adult survival and reproduction) in diverse boreal forest soils and horizons (e.g., organic and mineral horizons). Varying soil characteristics were also evaluated to determine their influence, if any, on these toxicological endpoints. Finally, the applicability of using *O. nitens* in the assessment of a toxic substance (boric acid) in forest soil was investigated. All tests (including statistical analyses) were conducted by J. Princz, with analytical support derived from a commercial laboratory.

[1] Princz JI, Behan-Pelletier VM, Scroggins RP, Siciliano SD. 2010. Oribatid mites in soil toxicity testing: the use of O*ppia nitens* (C.L. Koch) as a new test species. Environmental Toxicology and Chemistry, 29(4): 971-979.

3.2. Abstract

Few soil invertebrate species are available for the toxic assessment of soils from boreal or other northern ecozones, yet these soils cover the majority of Canada's landmass as well as significant portions of Eurasia. *Oppia nitens* (C.L. Koch) is an herbivorous and fungivorous oribatid mite found in soil throughout Holarctic regions, including Canada. Soil tests using *O. nitens* were performed using 15 different forest soil types and horizons to investigate test variability in adult survival and reproduction. Adult survival (86.1 ± 1.1%) was consistent across soil types, with a coefficient of variation (CV) of 15%. However, reproduction varied significantly, ranging from 2.9 (± 1.1) to 86.2 (± 11.7) individuals, with a corresponding CV of 118 and 30%, respectively. Of the soil factors assessed (NH_3, NO_3, pH, phosphorus, organic matter content, carbon:nitrogen, sand, silt, clay, and sodium adsorption ratio), soil organic matter (OM) explained 68% of the variation observed for reproduction. Increasing the OM using *Sphagnum* sp. peat moss resulted in optimal reproduction at 7% OM (8% peat content) with the lowest variability (CV of 20%). When assessing the toxicity of a reference chemical, boric acid, the effect of peat amendment reduced lethality to adults with no observable difference on reproduction. The use of an age-synchronized culture reduced the test variability for reproduction relative to the use of unsynchronized cultures. *Oppia nitens* is a good candidate species for a standardized test design, with adult survival easily assessed in a relatively simple design. A long-term reproduction test with *O. nitens* will require the use of a synchronized population and, on occasion, OM amendment when testing soils with low organic matter content.

3.3. Introduction

The need for standardized and ecologically-relevant tests for the risk assessment of individual contaminants or contaminant mixtures in Canadian non-temperate soils was identified in a recent review by Römbke et al. (2006). The authors recommended ecologically-relevant species for consideration as potential toxicity test organisms. Oribatid (Oribatida = Cryptostigmata) mites were considered relevant for future consideration, but the possibility of realistically using a representative species from this taxonomic group was challenged because of limited established methods, and slower developmental cycles observed to date with oribatid

species. Although oribatid mites are recognized as significant contributors to soil processes, they are under-represented in terrestrial ecotoxicological studies.

Oribatid mites are free-living and typically the most abundant and diverse microarthropod present in boreal forest soils (Crossley and Bohnsack, 1960; Walter, 1985; Heneghan et al., 1999), significantly contributing to nutrient cycling (e.g., mineralization) (Singh et al., 1996; Johnston and Crossley, 2002), and soil formation (Coleman et al., 2004). Their abundance, diversity, ability to colonize different soil habitats and horizons, occupancy of varied trophic levels, reproductive habits (e.g., iteroparity, sexual and parthenogenetic reproduction) and dispersal strategies make them ideal for the assessment of soils (Behan-Pelletier, 1999). Oribatid mites typically exhibit slow metabolism and development, low fecundity and long life spans with a low capacity for population increase (Lebrun and van Straalen, 1995; Norton and Behan-Pelletier, 2009). These characteristics, coupled with limited dispersal capacity (Hansen, 1999), contribute to their vulnerability to soil disturbances.

Oribatid mites have been used in the assessment of soil contamination, as indicators of soil quality (Andrés, 1999; Al-Assiuty et al., 2000), and susceptibility to metal (Denneman and van Straalen, 1991; Siepel, 1995; Skubała and Kafel, 2004), pesticide (Prinzing et al., 2002; Beck et al., 2004), and hydrocarbon pollution (Blakely et al., 2002). The use of oribatid mites in ecotoxicological studies was reviewed (Lebrun and van Straalen, 1995), with subsequent development of a lethality and reproduction laboratory soil assay using the parthenogenetic species *Platynothrus peltifer* (C.L. Koch, 1839) (van Gestel and Doornekamp, 1998). However, the standardization of this method was limited by the need to use field-collected specimens because of difficulties in the establishment and maintenance of laboratory cultures. Although the tests were effective at discerning effects on reproduction after exposure to contaminated soil, the tests were compromised by high adult mortality, and were lengthy (e.g., 6 to 12 weeks for reproduction endpoint) to accommodate the species' long development cycle (e.g., > 150 d to maturity) (van Gestel and Doornekamp, 1998). More recently, studies have incorporated the use of *Archegozetes longisetosus* (Aoki, 1965) as a laboratory test species (Seniczak and Seniczak, 2002; Köhler et al., 2005; Seniczak, 2006; Seniczak et al., 2006; Heethoff et al., 2007; Seniczak et al., 2009) as this species is also parthenogenetic, easily cultured under laboratory conditions,

and characterized by a short generation time with relatively high fecundity (Heethoff et al., 2007). However, this species is limited to a pan-tropical distribution; therefore, the relevance of this species to non-tropical habitats is questionable, particularly when assessing soils of boreal and northern regions.

Despite these studies, the number of ecotoxicity tests that incorporate oribatid mites in the assessment of contaminated soils pales in comparison with that of other soil invertebrates (e.g., lumbricids, enchytraeids, Collembola, isopods, gastropods). The purpose of the present study was to evaluate the soil mite *Oppia nitens* (C.L. Koch, 1836) as a suitable test species for the development of a mite toxicity test for use with a variety of soil types.

Oppia nitens is a brachypyline species belonging to the family Oppiidae, the largest oribatid family with approximately 1000 species comprising 129 genera (Ohkubo, 2001; Subías LS 2009: http://www.ucm.es/info/zoo/Artropodos/Catalogo.pdf). This species is known to occur in the upper horizons of forest soils, rich in mycelium and decayed organic matter (Seniczak, 1975a). *Oppia nitens* is considered to be a polyphagous fungivore, but does exhibit selective feeding preferences including lichens, raw humus and carrion (Seniczak, 1975b; Seniczak and Stefaniak, 1978).

The developmental (Sengbusch and Sengbusch, 1970) and morphological stages (Seniczak, 1975b) from nymph to adult have been characterized; maturation time is temperature dependent, ranging from 21 to 28 d at 23°C (Stefaniak and Seniczak, 1981; Yu et al., 1997) to 45 to 46 d at 20°C (Sengbusch and Sengbusch, 1970). There is conflicting information regarding reproductive mode (i.e., sexual or parthenogenetic); however, observations of spermatophores under laboratory conditions (Stefaniak and Seniczak, 1981) are suggestive of sexual reproduction.

The specific objectives of the present study were to determine which soil characteristics might positively influence the reproductive success of *O. nitens*; to compare the importance of organic matter content to adult and immature stages; to compare the response between age-

synchronized and unsynchronized test populations; and to determine the applicability of using this species in the assessment of reference soils amended with boric acid.

3.4. Materials and Methods

3.4.1. Culturing

The original culture, already laboratory reared for 20 years, was supplied by R. A. Norton and V. M. Behan-Pelletier, and has the following provenance: Canada, Ontario, Ottawa, Central Experimental Farm, 45º22.45'N 75º43.52'W, vi.1986 (V. M. Behan-Pelletier) from litter at the edge of a field. Specimens were reared at 20 ± 3°C in 125-ml glass mason jars lined with an 8:1 ratio of plaster of Paris and charcoal substrate. The substrate was moistened once per week with deionized water, and grains of Baker's yeast were added as a food source as required. The quantity of yeast added was based on the number of grains consumed weekly. Egg deposition was limited to the sides of the culture vessels or underneath the grains of yeast; eggs were laid either in single or in small clusters (e.g., 3-4 eggs). Transfer of the adults to fresh culture substrate occurred every 3 months, and transfer to the new substrate generally stimulated a round of egg production within the culture.

3.4.2. Age-synchronization

Age-synchronization was achieved by the transfer of tritonymph instars to a new container, where they were allowed to moult and mature into adults within 5 to 7 d of one another. Prior attempts at age-synchronization using adults were not successful as adults tended to lay eggs under the yeast grains. Upon removal of the adults, the yeast began to mould, and the eggs did not hatch. Similarly, when food grains were removed, and the eggs placed back into the culture vessel, the adults' feces tended to grow mould; therefore, of the fraction of eggs that hatched, only a small number of individuals survived. This demonstrated the importance of adults in the control of mould and fungal formation within the culture vessels.

Age-synchronized individuals were used for the boric acid studies only. All age-synchronizations were based on harvesting the tritonymph instars as described above, rather than eggs.

3.4.3. Survival and reproductive tests

3.4.3.1. Reference soil tests

Adult survival and reproductive success (measured as the total number of juveniles produced), using organisms from an unsynchronized test culture, were evaluated using various uncontaminated field-collected reference soils, in addition to a formulated artificial soil; selected characteristics of the soils are presented in Table 3.1, and full site descriptions are provided in Appendix A. The artificial soil was formulated according to EC (2007), and comprised 70% silica sand, 20% kaolin clay, 10% air-dried (sieved using a 2-mm mesh sieve) *Sphagnum* sp. peat moss and deionized water; the pH of the soil was adjusted using calcium carbonate. The soils were collected from the following locations in Canada: New Brunswick: 47°56'N, 66°31'W and 47°44'N, 66°32'W; Ontario: 45°39'N, 77°38'W; Saskatchewan: 53°40'N, 104°12'W and 53°34'N, 104°10'W; and Alberta: 54°45'N, 115°31'W and 56°21'N, 117°11'W. Soil horizons within the sites were collected separately and evaluated as distinct soil samples. Ten adults were added to each of five replicates containing 15 ml of soil (3.5 cm depth) in a 50-ml glass Schell vial, mixed to its optimal moisture content between 45 to 85% of the soil's water holding capacity (measured according to EC (2007); only three replicates were used for the SH06 and PR06 soils (refer to Table 3.1 and Appendix A for further details on these soils). The test vials were incubated at 20°C at 16:8 h light:dark (>800 lux) for 28 d. Thereafter, the adult and immature mites were heat-extracted from the soil using a Tullgren apparatus (Science Applications International Corporation, Ottawa, Ontario, Canada) and manually counted. As an additional step, the heat-extraction efficiency was checked for each test vessel by examining and counting any organisms from the empty test vessels and heat-extracted soils using water flotation; both adult and immature mites floated to the top of the water surface.

Table 3.1. Selected physical and chemical characteristics of field-collected reference soils; individual soil horizons within the sites were collected separately and evaluated as distinct soil samples (refer to the Materials and Method's section for location co-ordinates).

Soil[†]	NH_3 (mg kg^{-1})	NO_3 (mg kg^{-1})	P (mg kg^{-1})	pH	OM[‡] (%)	C:N	SAR	MC[§] (%)	Sand (%)	Silt (%)	Clay (%)	Soil texture[#]	Soil classi-fication	Source[‡‡]
Artificial soil (AS)	10	1.0	6	7.5	6.2	9.7	0.3	70	76	8	16	SL	n/a[††]	In-house
NB07-2011-FH/A	426	7.0	29	4.0	53	30	2.4	85	82	12	6	LS	Podzol	NB
NB07-2011-B	4.0	24	14	4.5	16	25	1.1	70	75	19	6	SL	Podzol	NB
NB07-2009-FH/A	783	3.0	99	4.7	77	24	1.8	120	79	1	20	SCL	Gleyed Humic-ferric Podzol	NB
NB07-2009-B	19	9.0	18	4.6	11	16	1.2	74	62	28	10	SL	Gleyed Humic-ferric Podzol	NB
ON07-Ahe	128	1.0	16	4.6	58	33	2.0	71	82	12	6	LS	Podzol	ON
ON07-Ae	4.0	1.0	2	4.6	2.1	26	2.8	54	88	6	6	LS	Podzol	ON
ON07-Bf	2.0	1.0	2	5.8	2.2	21	2.4	47	86	6	8	LS	Podzol	ON
PR06-Ah	2.0	15	17	7.1	9.5	15	1.2	62	51	43	6	SL	Rego Dark Grey Cherno-zem	AB
SH06-Of/Oh	114	3.0	28	3.9	68	17	0.9	73	-[¶]	-[¶]	-[¶]	Peat	Rego Humic Gleysol	AB
SK05-1-LFH	193	5.0	36	6.2	47	21	0.02	72	69	16	15	SL	Dark Grey Luvisol	SK
SK05-1-Ahe	21	1.0	32	5.3	12	0.8	0.1	59	49	42	10	L	Dark Grey Luvisol	SK
SK05-1-Bt	4.0	1.0	9	5.2	2.4	0.3	0.2	50	43	49	8	L	Dark Grey Luvisol	SK
SK05-2-LFH	80	3.0	17	6.5	22	4.0	0.4	60	77	6	17	SL	Orthic Eutric Brunisol	SK

Soil[†]	NH_3 (mg kg^{-1})	NO_3 (mg kg^{-1})	P (mg kg^{-1})	pH	OM[‡] (%)	C:N	SAR	MC[§] (%)	Sand (%)	Silt (%)	Clay (%)	Soil texture[#]	Soil classi-fication	Source[‡‡]
SK05-2-AB	10	1.0	4.0	5.8	2.1	0.6	0.1	45	84	14	2	LS	Orthic Eutric Brunisol	SK
Analytical method	2N KCl extrac-table	2N KCl extrac-table	$NaHCO_3$ extrac-table	1:1 water method	Loss on igni-tion			(EC, 2007)	Particle size distribution (filter candle system)			n/a	n/a	n/a

[†]Further details on soil codes, and horizons collected (individually or mixed) for these soils are provided in Appendix A
[‡]Organic matter content
[§]Moisture content presented as a percentage of the soil's water holding capacity
[¶]Analysis not possible; the sample was peat, rather than soil (i.e., sand, silt or clay)
[#]SL = sandy loam; LS = loam sand; SCL = sandy clay loam
[††]not applicable
[‡‡]NB = New Brunswick; ON = Ontario; AB = Alberta; SK = Saskatchewan

3.4.3.2. Organic matter and soil structure amendment

The influence of organic matter amendment on organism survival and reproduction was evaluated by amending a low organic soil using two soil conditioners. Reference soil SK05-1-Bt, with low organic matter content (2.4%), was selected for the present study. The organic matter content of the soil was increased by the addition of *Sphagnum* sp. peat moss (sieved using a 2-mm mesh sieve) on a dry weight basis, followed by thorough mixing with the reference soil. Vermiculite, an inert and pH-neutral substrate, was also used as a physical alternative to peat to determine whether soil structure per se could be an influential factor for juvenile production given that oribatid mites would tend to lay eggs within and between particles for protection of the eggs and progeny in nature. The vermiculite was washed three times using deionized water before addition to the soil, but the peat was not. A sufficient mass of each was used to achieve the following nominal treatments: 2 (i.e., no amendment), 4, 8, 16, 32, and 64% peat or vermiculite content. Ten adults were added to each test vessel and the test was conducted the same as described for the reference soil tests.

3.4.3.3. Soil toxicity testing

The toxicity of boric acid (H_3BO_3), a standard reference toxicant (EC, 2007) was evaluated using the SK05-1-Bt soil in the presence or absence of peat added as a soil conditioner. To reduce the influence of acidity, the peat was washed three times with deionized water prior to soil amendment; the final soil pH was 4.4, which was less than the non-amended soil (pH = 5.2). The quantity of peat added was sufficient to raise the organic matter content of the soil to 6.4% (8% peat content). Once the peat was added, the soil was equilibrated for three days prior to amending with boric acid. Three separate tests were prepared: a peat-amended boric acid test using organisms from an age-synchronized test culture; a peat-amended boric acid test using organisms from an unsynchronized test culture; and a non-amended boric acid test using organisms from an unsynchronized test culture. The boric acid was prepared and added to the soil via a stock solution; the following nominal concentrations were prepared: 0, 60, 100, 160, 256, 410, 655, and 1050 mg boric acid per kg of oven dry soil (mg kg^{-1}) for the peat-amended tests; and 0, 30, 60, 100, 160, 256, 410, and 655 mg kg^{-1} for the non-amended test. Ten adults

were added to each of three and four replicates for the peat-amended tests using organisms from the age-synchronized and unsynchronized test cultures, respectively. Twenty adults were added to each of four replicates in the non-amended test because of the anticipated lower reproduction as observed in initial studies. The tests were conducted using the test conditions stated above.

3.4.4. Statistical analysis

Stepwise multiple-regression was performed using SPSS 16.0 to evaluate significant relationships between soil characteristics [ammonia (NH_3), nitrite (NO_2), pH, phosphorous (P), organic matter (OM) content, carbon:nitrogen (C:N) ratio, sand, silt, clay and sodium adsorption ratio (SAR)] and biological response data [adult survival and reproduction (measured as the total number of juveniles produced)]. All data, except for soil pH, were transformed using a $\log_{10}(x+1)$ transformation for the regression analysis. The effect of peat or vermiculite amendment on adult survival and reproduction were assessed using analysis of variance; if the assumptions for normality and homoscedasticity of residuals were not met, the non-parametric test, Kruskal-Wallis, was used (EC, 2005a). If a significant effect, relative to the control response, was detected, pair-wise comparison analysis was employed, depending on whether parametric (e.g., Fisher's least significant difference (LSD) test) or non-parametric (e.g., Kruskal-Wallis) analyses were required. The LC50 (concentration causing 50% mortality) for adult survival was estimated using probit analysis, and the toxicity estimates compared using the Litchfield-Wilcoxon method (EC, 2005a). The IC50 (concentration causing 50% inhibition in reproduction) for reproduction was estimated using nonlinear regression analysis (EC, 2005a); however, although the assumption of normality was consistently met, the assumption of homogeneity of variance was not. Therefore, the data were reanalyzed using linear interpolation, and the expanded confidence limits (CL) were used because there were less than seven replicates per treatment (EC, 2005a). Statistical significance between reproduction estimates was determined through a comparison of confidence intervals (EC, 2005a) as well as using the generalized likelihood ratio test. The generalized likelihood ratio test yielded the same comparison, but was not cited as the assumptions for nonlinear regression models were not met, and linear interpolation did not yield equations for the concentration-response curves.

3.5. Results

3.5.1. Reference soil tests

For tests with organisms from an unsynchronized test culture, *Oppia nitens* adult survival was consistent among the various soils and horizons, with an overall mean percent survival of 86.1% with a standard error (SE) of 1.1% and a coefficient of variation (CV) of 15.0%. However, *O. nitens* reproduction varied significantly across the soils, with mean total juvenile production ranging from 2.9 (SE 1.1) individuals with a CV of 117.7% in the ON07-B horizon to 86.2 (SE 11.7) individuals with a CV of 30.3% in the NB07 2011-Ah horizon (Fig. 3.1). Overall, the mean coefficient of variation across the reference soils and various horizons for reproduction was high at 117.5%.

Of the soil characteristics assessed using multiple regression analysis (NH_3, NO_3, pH, P, OM, C:N, sand, silt, clay, and SAR (Table 3.1), none contributed to variations in adult survival. However, organic matter content could be used to explain 68% of the variability in reproduction ($r^2 = 0.68$; $p < 0.001$) (Fig. 3.2). No other soil characteristics were found to significantly contribute to reproduction. The resultant linear regression equation yielded: log(juveniles + 1) = 0.46 + 0.62*log(OM + 1).

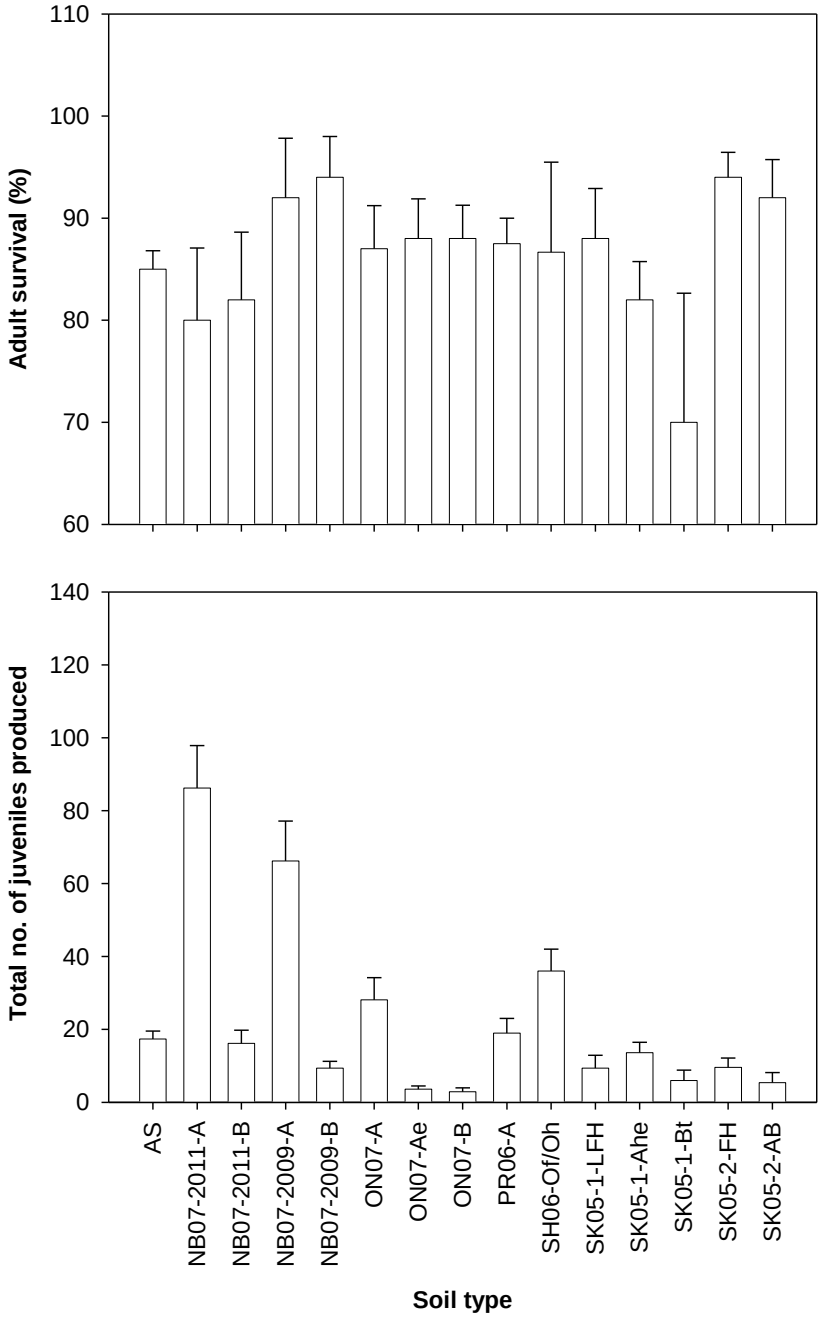

Fig. 3.1. Mean (± standard error) *Oppia nitens* adult survival and total juvenile production upon 28 d of exposure to several reference forest soil horizons and a formulated artificial soil. Between 1 and 2 separate tests were performed for the field soils, with 3 to 5 replicates per test; 43 tests were performed with formulated artificial soil.

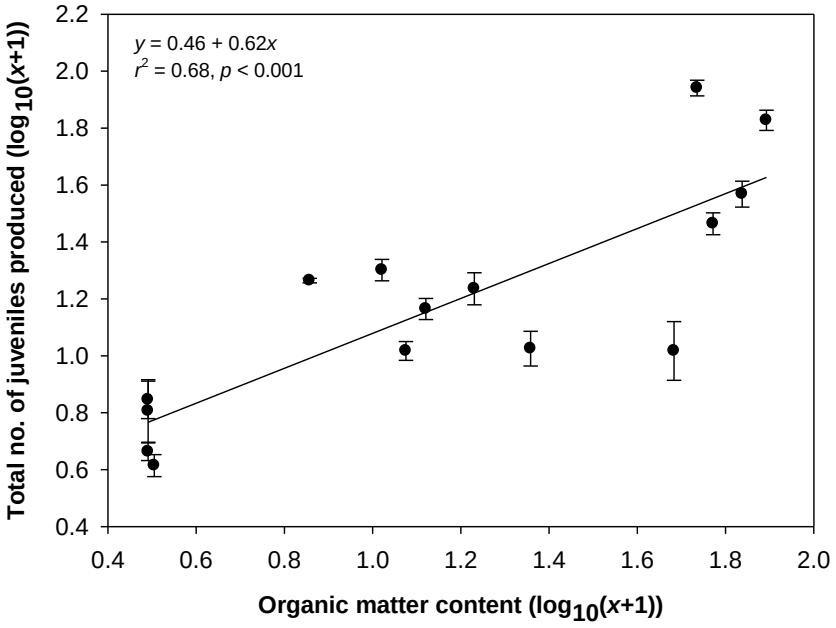

Fig. 3.2. Mean (± standard error) *Oppia nitens* total juvenile production as a function of organic matter content (%) in control tests using several reference forest soil horizons and a formulated artificial soil. The regression line was fit based on the results of stepwise multiple linear regression to determine significant soil characteristics affecting reproduction. Regressed data were transformed using a $\log_{10}(x+1)$ transformation.

3.5.2. Organic matter and soil structure amendment

The influence of organic matter content on adult survival and reproduction was assessed using the SK05-1-Bt soil horizon (2.4% OM) which had low reproduction (mean of 6.0 (SE 2.8) individuals) and high variability among test replicates (CV = 106.1%). The addition of peat to increase the organic matter content did not affect adult survival, but reproduction significantly ($p < 0.02$) increased with 4, 8, and 16% peat content. The greatest reproduction occurred at 8% peat content (equivalent to 7.0% OM) with a mean total juvenile production of 59.3 (SE 5.8), with the lowest CV of 19.5% (Fig. 3.3). At higher peat contents, the variation increased by a factor of 2.3 to 5.1 with each subsequent treatment, possibly due in part to the treatment's accompanying soil pH (4.0 – 3.6), which declined with increasing peat content. Despite this variation, *O. nitens* was able to produce viable offspring at soil pH levels less than 4.0.

In the test using vermiculite, reproduction increased with increasing vermiculite concentration, but then significantly decreased ($p < 0.02$) relative to the control response when 16% or more vermiculite was present within the treatment. The greatest reproduction, although not significant relative to the control response, was highest at 8% vermiculite content (2.2% OM), but reproduction was less (mean of 29.3 (SE 16.9) individuals) and was more variable (CV = 115.8%) than that observed in the same treatment using peat as a soil amendment (Fig. 3.3).

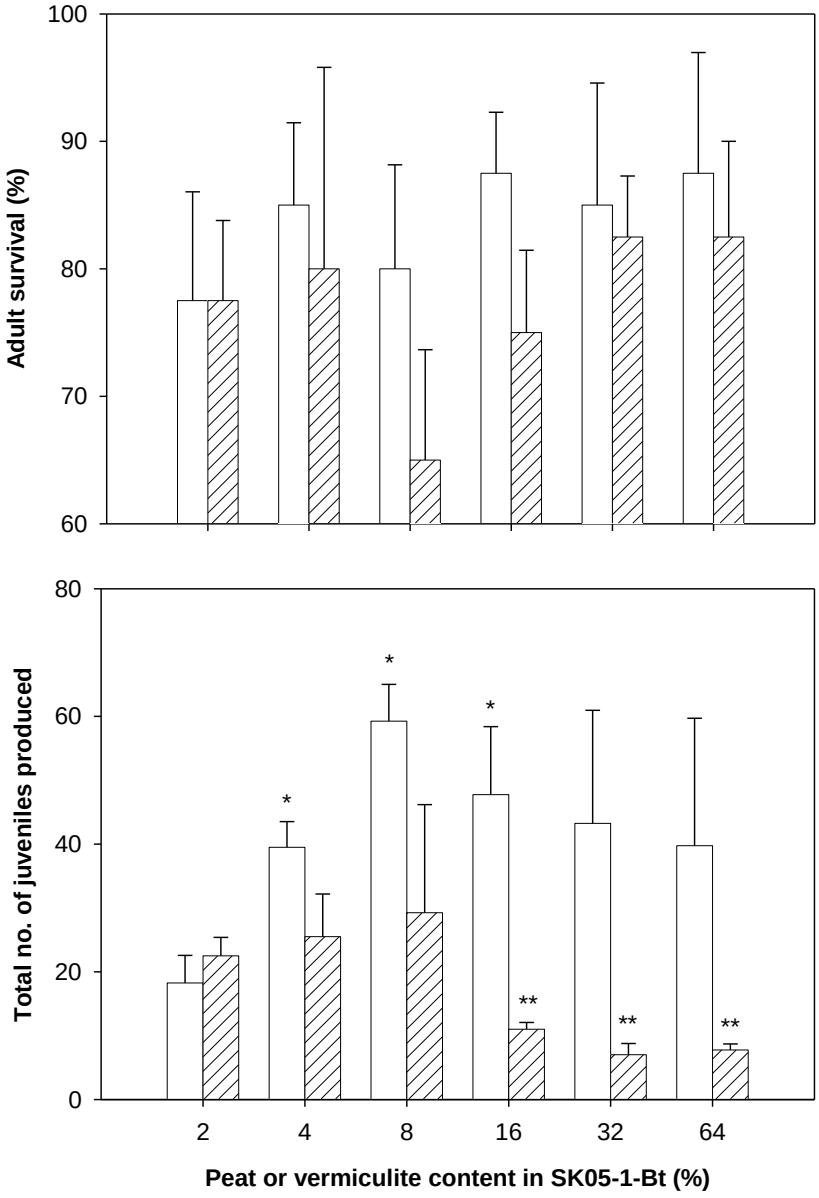

Fig. 3.3. Mean (± standard error) *Oppia nitens* adult survival (n = 3) and total juvenile production upon 28 d of exposure to SK05-1-Bt reference soil amended with either peat (white bars) or vermiculite (hatched bars) at varying concentrations. Stars denote a significance difference ($p < 0.05$) in response relative to the control treatment (no amendment representative of 2% natural organic matter); significance in the peat and vermiculite amendment are denoted by single and double stars, respectively.

3.5.3. Soil toxicity tests

The effect of peat amendment on the toxicity of a reference chemical (boric acid) was evaluated using organisms from both age-synchronized and unsynchronized cultures. For the unsynchronized test populations, adult survival was significantly less sensitive in the peat-amended soil (LC50 = 530 mg kg^{-1}, 95% CL: 424 – 624 mg kg^{-1}) when compared to the non-amended soil (LC50 = 250 mg kg^{-1}, 95% CL: 218 – 278 mg kg^{-1}) (Fig. 3.4). For *O. nitens* reproduction, there was no significant difference between the peat-amended soil (IC50 = 96 mg kg^{-1}, 95% CL: 16 – 152 mg kg^{-1}) when compared to the non-amended soil (78 mg kg^{-1}, 95% CL: 57 – 80 mg kg^{-1}) (Fig. 3.4).

In the peat-amended soils, adult survival was significantly less sensitive to the boric acid when using organisms from an age-synchronized culture (LC50 = 847 mg kg^{-1}, 95% CL: 705 – 990 mg kg^{-1}) relative to those from the unsynchronized culture (LC50 = 530 mg kg^{-1}, 95% CL: 424 – 624 mg kg^{-1}) (Fig. 3.5). Reproduction was comparable between the two treatments regardless of synchronization (age-synchronized: IC50 = 118 mg kg^{-1}, 95% CL: 91 – 138 mg kg^{-1}; unsynchronized: IC50 = 96 mg kg^{-1}, 95% CL: 16 – 152 mg kg^{-1}) (Fig. 3.5); however, the variation surrounding the IC50 was greater for the unsynchronized test population. In general, age-synchronization made little difference to the variability observed for adult survival, but decreased the test variability for reproduction (Fig. 3.6). Peat amendment in general increased *O. nitens* reproduction relative to the non-amended soils, regardless of age-synchronization. At the end of the test, reproduction in the peat-amended soil (mean of 93.5 (SE 33.1) individuals) was 2.4 times greater than in the non-amended soil (mean of 39.3 (SE 26.0) individuals) despite only having added 10 adults in the peat-amended soil test replicates, and 20 adults in the non-amended soil test replicates (Fig. 3.6).

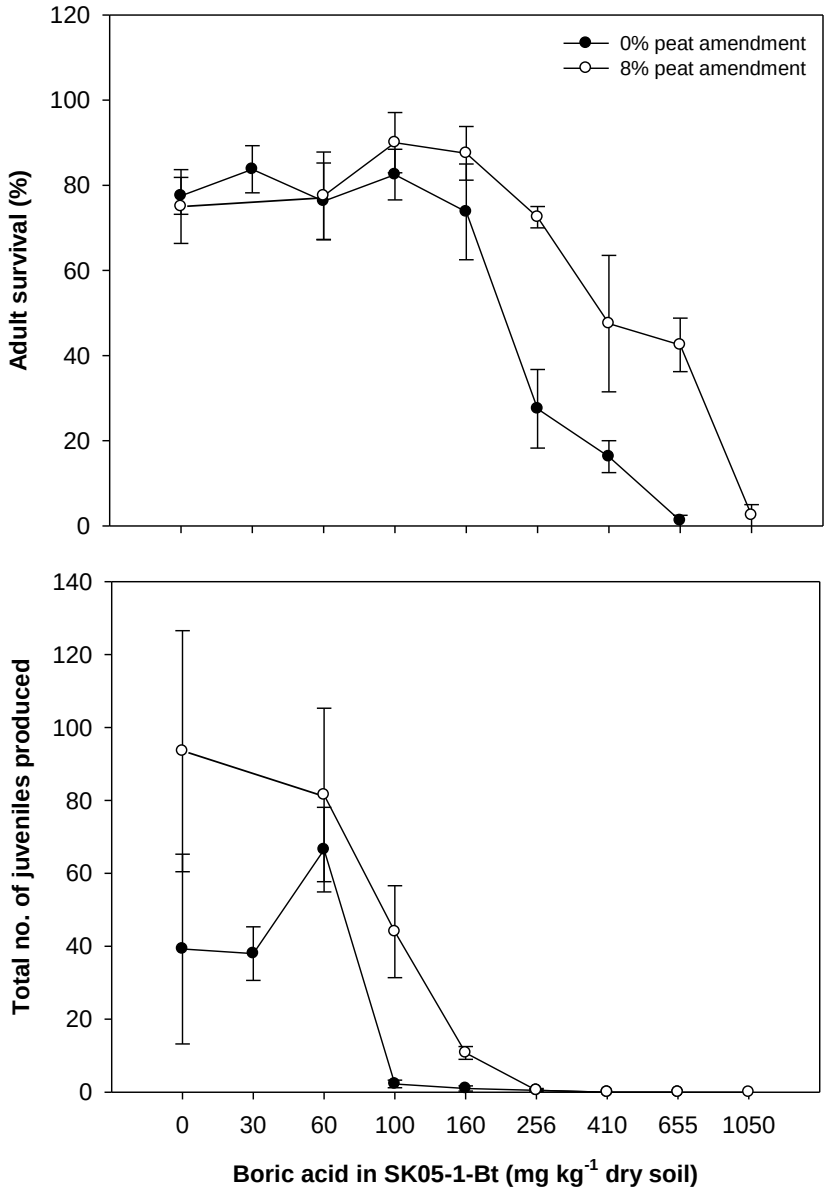

Fig. 3.4. The effect of boric acid on mean (± standard error) *Oppia nitens* adult survival and reproduction (i.e., the total number of juveniles produced) in non-amended (2% organic matter (OM)) (n = 3) and peat-amended (7% OM) (n = 10 adults) SK05-1-Bt reference soil.

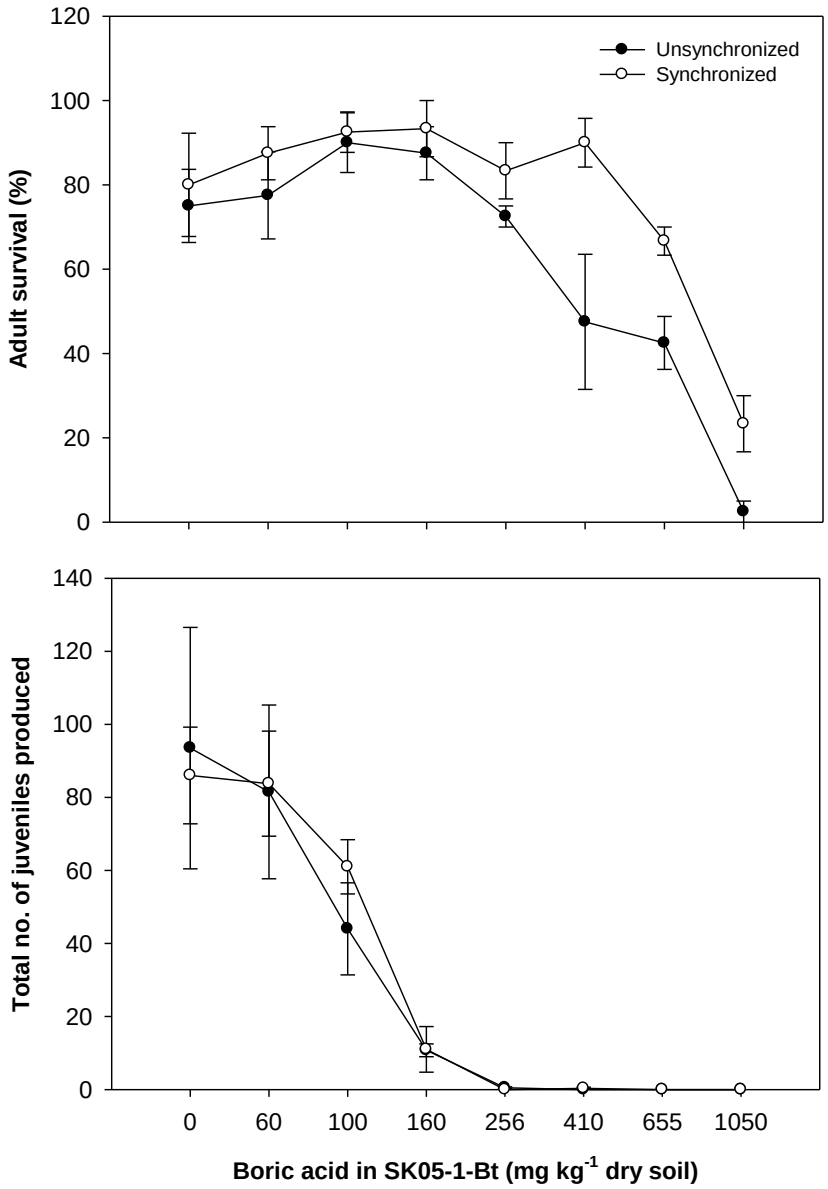

Fig. 3.5. The effect of boric acid on mean (± standard error) *Oppia nitens* adult survival (n = 3) and reproduction (i.e., total number of juveniles produced) in peat-amended (7% organic matter) SK05-1-Bt reference soil. Adult test populations were either unsynchronized or age-synchronized prior to the test.

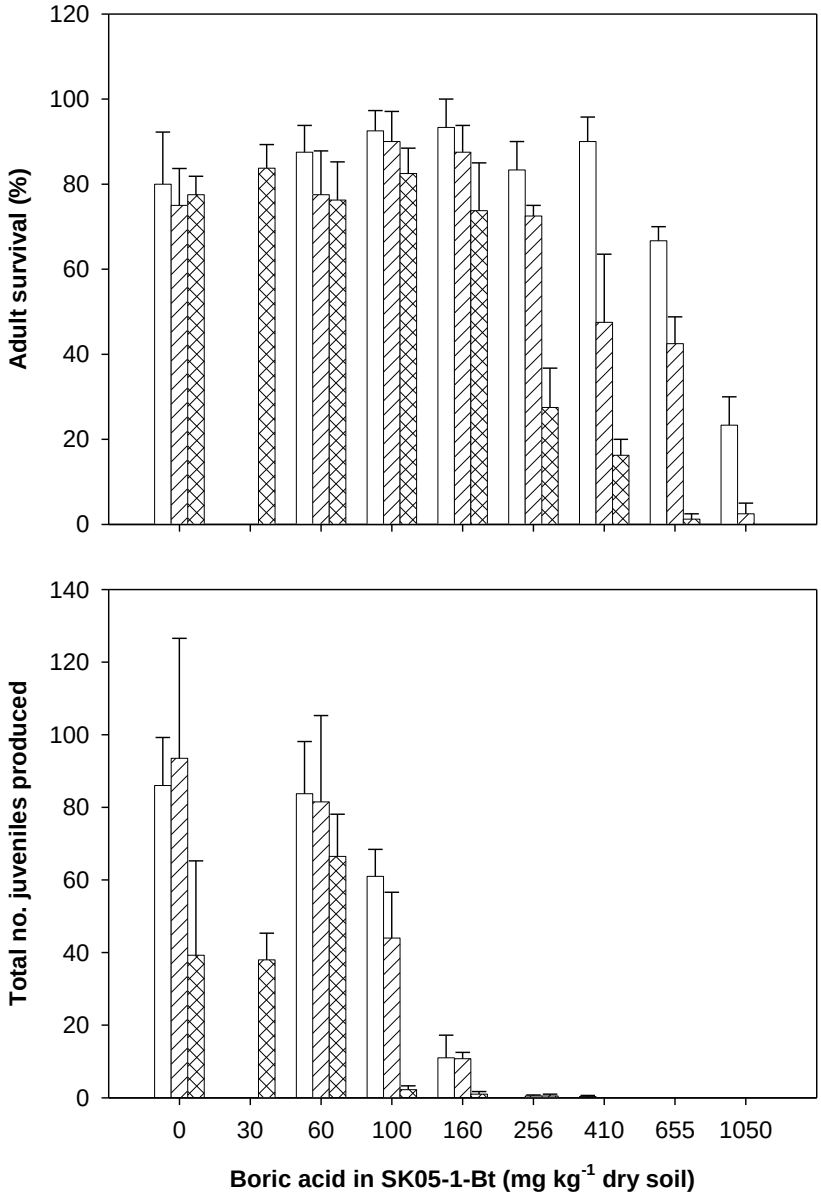

Fig. 3.6. The effect of boric acid in SK05-1-Bt (mg kg^{-1} oven dry soil) on mean (± standard error) *Oppia nitens* adult survival and total juvenile production. White bars represents the exposure of an age-synchronized test population (n = 3) to boric acid in SK05-1-Bt reference soil amended with 8% peat. Hatched bars represents the exposure on an unsynchronized test population (n = 10 adults) to boric acid in SK05-1-Bt amended with 8% peat. Cross-hatched bars represents the exposure of an unsynchronized test population (n = 20 adults) to boric acid in SK05-1-Bt with no peat (0%) amendment.

3.6. Discussion

In the present study, soil tests were completed using multiple field-collected (i.e., uncontaminated) reference forest soils and horizons, as well as chemically-amended soil samples. To our knowledge, only one other study has used *O. nitens* as a test species. The toxicity of *Bacillus thuringiensis* toxins in transgenic cotton and potato was assessed (Yu et al., 1997) whereby ten adults were exposed to contaminated leaf discs or milled leaves in 0.7 g of autoclaved soil. The effect of dietary exposure only was evaluated on adult survival and reproduction, and no adverse effects were detected. These authors used cadmium as a positive control, but did not report the effects of the metal on either adult survival or reproduction.

Lebrun and van Straalen (1995) advocated for the consideration of oribatid mites in soil ecotoxicological testing, while recognizing their diversity and structural and functional complexity within soil ecosystems. The need for method standardization was identified with efforts directed towards the development of a lethality and reproduction assay using the parthenogenetic species *Platynothrus peltifer* (van Gestel and Doornekamp, 1998). The resultant methods included both dietary exposure and soil exposure studies. An evaluation of copper, sodium salt of linear alkyl benzenesulphonate (LAS), and dimethoate demonstrated increased toxicity for copper and LAS when *P. peltifer* was exposed to contaminated test soil relative to dietary exposure on a plaster of Paris substrate (van Gestel and Doornekamp, 1998) no difference was observed for dimethoate. The study demonstrated the need for a soil exposure test system to take into account other exposure pathways (e.g., soil pore water) in the evaluation of soil contaminants. The use of a soil exposure system also allows for comparison of species sensitivity to other standard soil invertebrate toxicity tests.

In addition to *P. peltifer*, several laboratory-based assays have used the pan-tropical species, *Archegozetes longisetosus* (Seniczak and Seniczak, 2002; Köhler et al., 2005; Seniczak 2006; Seniczak et al., 2006; Heethoff et al., 2007; Seniczak et al., 2009). This species is parthenogenetic, easy to rear under laboratory conditions and highly fecund (Köhler et al., 2005). In a study to evaluate the fecundity of *A. longisetosus*, it was demonstrated that the rearing of a single mature adult yielded 61 eggs within the culture box (5 cm^2) with a mean number of F1

adults per initial female of 55.3 (Seniczak, 2006). The fecundity of *P. peltifer*, based on the reported study (van Gestel and Doornekamp, 1998) equates to approximately 5 juveniles per adult in a soil test; in our studies, the fecundity of *O. nitens* was approximately 2.7 juveniles per adult, although the test duration was much less than that reported for *P. peltifer*. Toxicological studies using cadmium have demonstrated effects on *A. longisetosus* longevity, development and fecundity (Seniczak and Seniczak, 2002; Köhler et al., 2005; Seniczak et al., 2006; Heethoff et al., 2007; Seniczak et al., 2009) under standard laboratory conditions. However, the studies only involved dietary exposure on a plaster of Paris substrate. *Oppia nitens* as a test species, exhibits the ability for sustained laboratory cultures, a much shorter life cycle relative to *P. peltifer*, resulting in a shortened test duration (4 weeks) with low adult mortality in a soil test (e.g., >70% mean survival across all soil types, with 85% survival in artificial soil).

In contrast to dietary exposure studies, the exposure of *O. nitens* to several soil types and horizons allowed us to evaluate which soil characteristics might influence the survival and reproduction of this species in the absence of soil contaminants. Of all soil characteristics evaluated (NH_3, NO_3, pH, P, OM, C:N, sand, silt, clay, and SAR), only organic matter content affected *O. nitens* reproduction. Using a sub-optimal soil (i.e., lower organic matter content and lower juvenile production), we found that if the organic matter content was increased, reproduction increased. Reproduction was observed to be optimal at an organic matter content (amended as *Sphagnum* sp. peat moss) of 6 to 7%, with the lowest replicate variation (Fig. 3.3). When vermiculite was used as a physical substrate in contrast to peat, reproduction was also greatest at 8% vermiculite content (2.2% OM content), but reproduction levels were much lower than that observed with the peat amendment, and subsequently decreased with increasing vermiculite content. In the present study, the addition of peat as an organic substrate was more influential on reproduction, and there was no benefit to reproduction when vermiculite was added as an alternative substrate.

Maraun and Scheu (2000) reported that oribatid mite community structure is predominantly driven by humus form, rather than other parameters such as soil acidity, humidity, and temperature. Thus, it is not surprising that organic matter content affects juvenile production as with increased organic matter in the litter and humus layers, an increase in fungal biomass, a

primary food source for the mites, would also occur. Despite being provided with a constant laboratory food source during the test, food preferences for certain fungal species are known to exist [e.g., (Behan-Pelletier and Hill, 1983; Hubert et al., 2001; Schneider and Maraun, 2005; Koukol et al., 2009)] and, if present in the test soils, could contribute to an increase in energy resources and reproduction. However, this does not explain why reproduction was lower in some of the other organic forest soil horizons (e.g., SK05-1-LFH, SK05-2-LFH; Fig. 3.1), though it is possible that organic matter quality differed between soils with similar organic matter content, given that the test soils were collected from various regions across Canada. Organic matter content and quality varies and is limiting across horizons rather than within horizons (Klironomos and Kendrick, 1995). As organic matter content was found to affect reproduction, the individual soil horizons within a site should be taken into consideration when interpreting test results. This is particularly important when evaluating the results of a toxicity test where the performance of the test organism in the contaminated soil is being compared with that in the non-contaminated soil.

Although the addition of organic material increased overall reproduction and reduced test variability, the addition of organic matter (or in this case, peat) to a soil can change the bioavailability of a freshly-amended contaminant to a soil organism thus affecting toxicity. In our studies, the toxicity of boric acid in the peat-amended soil was two times less than that observed in the non-amended soils for adult lethality. For reproduction, no significant difference was evident between the treatments, however, the lack of effect could have been a result of the observed high test variability. It is uncertain whether such a difference would be present in an aged contaminated field soil, where the contaminants have an established equilibrium and are generally recalcitrant. The addition of organic material would not be warranted for evaluating effects on adult lethality, as adult survival was unaffected in the test soils where the organic matter content varied from 2.1 to 67.8%.

The performance of *O. nitens* in the reference soils suggests that *O. nitens* can tolerate a relatively large soil pH range (e.g., 3.9 to 7.5 for the field-collected soils). When evaluating the influence of the soil characteristics on reproduction, soil pH was not a significant influential factor ($p = 0.293$) on the observed response variability. For some of the test soils, particularly

horizons containing organic matter, reproduction was evident even though the soil pH was <4.0 (e.g., SH06-Of/Oh soil pH = 3.9 and NB07-2011-A soil pH = 4.0; Fig. 3.1). In the peat amendment study, increased reproduction was observed when the organic matter content was increased, although the pH of the soil decreased from 5.2 to 4.4. In general, the effect of soil pH on oribatid mite abundance seems to be species specific with some species exhibiting narrow tolerance thresholds and some having relatively wider tolerances (Hågvar and Abrahamsen, 1980; van Straalen and Verhoef, 1997).

In addition to organic matter content, we also found that the use of an age-synchronized culture reduced the reproduction variability for this species. Age-synchronization reduces test variation by standardizing the developmental and physiological stage of the organism (Kula and Heimbach, 1998), which can greatly influence their susceptibility to a contaminant. Immature forms of *O. nitens* lack the developed sclerotized integument present in adults and therefore experience different degrees of exposure via different pathways (e.g., dermal absorption through the integument). Differences in sensitivity between life stages are in part explained by stage-specific surface to volume ratio, which is larger for smaller immature mites, when compared with larger adult life stages (Heckmann et al., 2005).

4. THE TOXICOKINETICS OF A MODEL COMPOUND – PHENANTHRENE – TO *OPPIA NITENS*

4.1. Preface

The first study successfully demonstrated the use and applicability of the oribatid mite, *Oppia nitens*, as a suitable soil toxicity test species. Research also demonstrates the sensitivity of oribatid mites to hydrocarbon pollution in soil (Parmelee et al., 1993; Lebrun and van Straalen, 1995; Erstfeld and Snow-Ashbrook, 1999; Blakely et al., 2002), some of which may include various types of PAHs. Polycyclic aromatic hydrocarbons are also likely to partition within the organic components of soil, particularly horizons rich in organic matter (Jager et al., 2000; Krauss et al., 2000; Zhang et al., 2006). Because oribatid mites are known to primarily inhabit organic-rich horizons and soils, it made sense to evaluate the toxicokinetics of a model PAH (phenanthrene) within *O. nitens*. The study evaluated the toxicity and bioaccumulation potential of phenanthrene to *O. nitens* in an artificial soil. All tests (including statistically analyses) were conducted by J. Princz, with analytical support derived from the Department of Soil Science (O.J. Owojori and S.D. Siciliano).

4.2. Abstract

Oribatid mites primarily inhabit the organic-rich horizons and soils, to which polycyclic aromatic hydrocarbons (PAH) can partition within. The effect and bioaccumulation potential of a model PAH, phenanthrene, was assessed to *Oppia nitens* in artificial soil. Phenanthrene was toxic to *O. nitens* adult survival (LC50 = 388 mg kg^{-1}, 95% confidence limits [CL]: 330 - 446) and juvenile production (IC50 = 95 mg kg^{-1}, 95% CL: 26 - 345) at levels similar to those observed by other soil invertebrates as reported in the literature. Exposure of *O. nitens* to phenanthrene resulted in consistent accumulation across time for the test concentrations assessed (i.e., 100, 200 and 400 mg kg^{-1}); however, steady-state was not reached during the four-week exposure period. Once transferred to clean soil, the organisms were able to eliminate the chemical; however, residual levels of phenanthrene were evident at the end of the elimination phase, possibly due to cuticular sorption processes. High replicate variability was observed throughout the uptake and elimination phases, and were likely a result of low sample size (n = 20 per replicate), although phenanthrene could none the less be detected at levels above the method detection limit. Bioaccumulation increased with increasing exposure concentration, but the resultant kinetic bioaccumulation factors (BAF_{kc}, corrected for lipid and soil organic carbon content) were low (<0.031), indicative of limited trophic transfer and biomagnification for this species.

4.3. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a wide-spread contaminant of concern resulting from emissions associated with combustion processes, and the processing of fossil fuels (Jager et al., 2000). The deposition of these materials into soil, either through atmospheric or direct application (e.g., spill), are also of concern as these hydrophobic substances partition to organic components within the soil, leading to the accumulation and persistence within soil horizons rich in organic matter (Jager et al., 2000; Krauss et al., 2000; Zhang et al., 2006). Contamination within the organic layers of forest soil is of significance, as these layers support diverse flora and fauna that contribute and maintain the overall soil's structure and function.

Among the myriad of soil invertebrates present, oribatid mites are an important presence within soils (Crossley and Bohnsack, 1960; Heneghan et al., 1999), significantly contributing to nutrient cycling (e.g., mineralization) (Singh et al., 1996; Johnston and Crossley, 2002), and soil formation (Coleman et al., 2004). Although recognized as significant contributors to soil processes, oribatid mites are under-represented in terrestrial ecotoxicological studies (Chapter 2). Oribatid mites are sensitive to hydrocarbon pollution in soil (Parmelee et al., 1993; Lebrun and van Straalen, 1995; Erstfeld and Snow-Ashbrook, 1999; Blakely et al., 2002). However, as the toxicity of petroleum hydrocarbons is dependent on petroleum source, soil type, and varies with species and endpoints (Salanitro et al., 1997), direct comparisons across studies and species are difficult.

The mode of toxic action of the PAHs is generally through nonpolar narcosis, resulting in non-specific disruption of cell membranes (Jager et al., 2000; Sverdrup et al., 2001). However, specific mechanisms of toxicity for PAHs have been observed that involve biochemical activation, oxidative stress and the creation of carcinogenic reactive electrophilic species or biochemically reactive metabolites; some metabolites may not be toxic, but are hydrophilized for excretion. The fate of PAHs in soil to oribatid mites is unknown; however, exposure studies using other soil invertebrate species demonstrate the ability for accumulation, but with low body residue amounts, indicative of the ability for biotransformation and subsequent elimination, with little tendency for biomagnification (Jager et al., 2000; van Brummelen et al., 1996a; van Brummelen et al., 1996b). Research suggests that the P450 system and subsequent metabolism of PAHs is less active in invertebrates than vertebrates (Jones et al., 2008), with reports of no change in enzyme activity nor the presence of metabolites in earthworms exposed to pyrene (Achazi et al., 1998; Brown et al., 2004; Jones et al., 2008). In contrast, Stroomberg et al. (2004) demonstrated that the earthworm *Eisenia andrei* could metabolize pyrene, but with much less efficiency than the Collembola, *Folsomia candida*, and the isopod, *Porcellio scaber*, and hence accumulated the PAH to a much higher degree. There are no studies that have evaluated P450-mediated detoxification of PAHs in oribatid mites, although P450 activity associated with the detoxification and resistance to acaricides has been demonstrated in other acarine species (e.g., Crampton et al., 1999; Pottleberge et al., 2008; Tirello et al., 2012).

Very few studies have evaluated the toxicity and accumulation of PAHs in oribatid mites; however, soil toxicity tests with benzo[a]pyrene (BaP) demonstrated no significant effects on adult survival or reproduction at concentrations >1,600 mg kg^{-1} for *Oppia. nitens* (Owojori and Siciliano, 2012). Further evaluation of body residues yielded no detectable levels after the 35-d exposure; however, the metabolic capacity of *O. nitens* was uncertain as residues were not measured throughout the test, only at test end. However, the toxicity results were comparable to other soil invertebrate species, in that low toxicity was observed in general for this compound (Sverdrup et al., 2007). As little data are available on the effect of PAHs in oribatid mites, the aim of this study was to evaluate the toxicity and bioaccumulation potential of another PAH, phenanthrene, in *O. nitens*, using a standard formulated artificial soil. It is hypothesized that the oribatid mites might confer a greater degree of sensitivity to PAHs, as they exhibit slow metabolism, low fecundity, long life-span and limited dispersal capacity (Lebrun and van Sraalen, 1995; Hansen, 1999; Behan-Pelletier, 1999), allowing for greater exposure durations in polluted soils. Phenanthrene was used as a model PAH, as it is mainly found within organic soil horizons (Cortet et al., 2006), is a common component of PAHs (Yu and Lin, 2005), and considered a health risk not only to humans, but to soil organisms alike. The toxicity, toxicokinetics and bioaccumulation potential of phenanthrene has also been studied in various soil organisms (Crouau et al., 1999; Sverdrup et al., 2001; Sverdrup et al., 2002a and 2002b; Cortet et al., 2006; Amorim et al., 2011), demonstrating its relevancy to estimating the ecological risk of PAHs within the soil compartment.

4.4. Materials and Methods

4.4.1. Cultures and age-synchronization

The test organisms were reared at 20 ± 3°C in 125-ml glass mason jars lined with an 8:1 ratio of plaster of Paris and charcoal substrate. The substrate was moistened once per week with deionized water, and grains of Baker's yeast were added as a food source as required. The test organisms were age-synchronized prior to use in a test; age synchronization was achieved by collecting newly-emerged adults within four days of moulting. These individuals were easily distinguishable by their light pigmentation (i.e., pink-brown versus dark brown) due to

incomplete mineralization and sclerotization of their integument. Once collected into a separate vessel, the adults were allowed to mature for approximately one week prior to introduction to the test soil.

4.4.2. Soil toxicity test

Phenanthrene (98%, Sigma-Aldrich) was added to the test soil via the use of a solvent carrier, acetone (>95%, Fisher Scientific), and thoroughly mixed. The solvent was then allowed to evaporate from the soil for 24 h, after which the test soil was hydrated with deionized water to attain an optimal moisture content of 70% of the soil's water holding capacity. The test soil consisted of artificial soil, which was formulated according to Environment Canada (2007), and consisted of 70% silica sand, 20% kaolin clay, 10% air-dried (sieved using a 2-mm mesh sieve) *Sphagnum* sp. peat moss, and deionized water; the pH of the soil was adjusted using calcium carbonate. A summary of the soil characteristics is presented in Table 4.1, with further details provided in Appendix B. The soil toxicity test was performed by preparing the highest test concentration, followed by the dilution of this test concentration with clean uncontaminated soil; nominal test concentrations included 0, 7.8, 16, 31, 63, 125, 250, 500, and 1000 mg kg^{-1} dry soil. A solvent control was included, using the same concentration of acetone used to prepare the phenanthrene-amended test soil.

Table 4.1. Summary of selected physical and chemical characteristics of the artificial soil.

Parameter	Units	Method Reporting Limit	Artificial Soil
pH			7.4
Total Organic Carbon	%	0.01	5.5
Organic Matter	%	0.1	7.6
Sand (>0.050 mm)	%	1	76
Silt (>0.002-0.050 mm)	%	1	12
Clay (≤0.002 mm)	%	1	12

Ten adults were added to each of five replicates containing 15 ml of artificial soil (3.5 cm depth) in a 50-ml glass Schell vial. The test vials were incubated at 20°C constant at 16:8 h light:dark (>800 lux) for 28 d, and Baker's yeast and deionized water were added weekly.

Thereafter, the adult and immature mites were heat-extracted from the soil for 48 h, and manually counted. As an additional step, the heat-extraction efficiency was checked for each test vessel by examining and counting any organisms from the empty test vessels and heat-extracted soils using water flotation; both adult and immature mites floated to the top of the water surface. Test endpoints included the 28-d mean adult survival, and reproduction, measured as the mean number of juveniles produced.

4.4.3. Soil bioaccumulation test

The bioaccumulation test design was based upon the Organization for Economic Cooperation and Development (OECD) guideline 317 for assessing bioaccumulation in terrestrial oligochaetes (OECD, 2010). The test consisted of two phases: an uptake phase (28 days) whereby *O. nitens* were exposed to three concentrations of phenanthrene in soil; and an elimination phase (21 days), whereby the test organisms (20 per replicate) were transferred to uncontaminated (clean) soil. Three test concentrations were evaluated: 100, 200 and 400 mg kg^{-1} dry soil. The phenanthrene was added to the soil using acetone solvent as a carrier; the soils were then evaporated for 24 h to remove the solvent; a solvent control was included with the tests. Once the soils were amended and the solvent evaporated, the soils were hydrated with deionized water to attain an optimal moisture content of 70% of the artificial soil's water holding capacity. Triplicate samples of soil and mites were collected throughout each phase of the tests for chemical analysis; samples were collected on days 0, 1, 4, 7, 14, 21 and 28 for the uptake phase, and on days 1, 7, 14, and 21 for the elimination phase. Control samples were sampled only at the beginning and end of the uptake phase, as well as at the end of the elimination phase, after transfer to clean soil at the same time as exposed organisms. The test vessels were the same as used in the soil toxicity test, along with the incubation test conditions (i.e., 20°C constant at 16:8 h light:dark (>800 lux)); the test soils were moistened with deionized water, and the test organisms provided with Baker's yeast on a weekly basis. Test measurements included adult survival at each sampling date; for the OECD (2010) guideline, test validity requires no more than 10% mortality of test organisms at the end of the uptake phase.

4.4.4. Chemical analysis

Soil and mite samples were frozen and stored at -20°C prior to analysis. The mites were not depurated prior to chemical analysis, but as the heat extraction was 48 h, some depuration likely occurred. Lipid analysis was not conducted, however, a mean lipid content of 13% was assumed, based on estimates provided by Convey (1992); this is the only published reference on oribatid mite lipid content, representing two Antarctic species, *Alaskozetes antarcticus* and *Halozetes belgicae*, of which lipid content was not seasonal dependent. The mites within a replicate were pooled, with three replicate values per time point.

Accelerated Solvent Extraction (ASE) was used to extract the phenanthrene from the soil and tissue. The soil was sub-sampled and transferred to pre-weighed screw cap vials, and lyophilized until completely dry; the mites were not lyophilized, but added to the ASE cells directly. For quality control, all of the samples were analyzed in triplicate, and every tenth sample was spiked with 250 µL of PAH spike solution. The spike was a 10^{-2} dilution of the standard PAH solution.

Once lyophilized, the soil was placed into pre-weighed ASE cells, containing a filter at the bottom; all parts were rinsed with hexane prior to use. The cell weights were measured prior to, and after addition of the soil to determine how much sample was being extracted; in the case of the mites, the number of mites added to each cell was recorded. The cells were then filled with clean Ottawa sand, and another filter placed on top. The cells were packed with a rod that was rinsed with acetone between samples. The lid was rinsed again with hexane, and placed tightly onto the cell. The cells were then loaded onto the ASE with labeled collection vials, with every run having one cell with only Ottawa sand to serve as a blank.

The samples were then extracted using a Dionex® Accelerated Solvent Extractor (ASE 200), which was run with an 80:20 mixture of hexane:dichloromethane, and the cells were extracted at 2030 psi and 150°C. The program had a heat-up time of 7 min, with a static time of 5 min. The cells were then purged for 1 min, with 2 cycles per cell and a 100% flush volume.

The collection vials were then evaporated to near dryness, resuspended in 2 mL acetonitrile, and filtered through a 0.45 µm filter into 2-mL amber HPLC vials labeled with the database number.

High Performance Liquid Chromatography coupled with Fluorescence Detection (HPLC-FD) was used to analyze the prepared samples for phenanthrene. The phenanthrene was detected by injecting a 10 µL sample onto a Varian PAH Pursuit (3 µm particle size, 100 mm length, and 4.6 mm inner diameter) column guarded with a Varian MetaGuard 3 µm C18 4.6 mm column. Acetonitrile and HPLC grade water were used as solvents, and the runs were 30 min in duration. The solvent flow was 1 mL per min with a set solvent gradient (Table 4.2), and the phenanthrene was detected using a programmed fluorescence detector (Table 4.3). After the analyses, the vials were recapped and stored at -20°C indefinitely. The detection limit for phenanthrene was 7 pg μL^{-1}.

Table 4.2. HPLC water and acetonitrile gradients.

Time (min)	Water (%)	Acetonitrile (%)
0.0	40	60
3.0	40	60
7.0	10	90
13.3	0	100
16.0	0	100
16.1	40	60
21.1	40	60

Table 4.3. Fluorescence detector settings.

Time (min)	Excitation wavelength (nm)	Emission wavelength (nm)
0.0	270	323
5.0	250	375
7.3	270	410
8.6	265	380
10.6	280	420
15.2	293	498

4.4.5. Statistical analyses

The LC50 (i.e., concentration causing a 50% effect) for adult lethality was determined using the Spearman-Kärber method of analysis. The IC50 (or 25) (i.e., concentration causing a 50 or 25% inhibition in juvenile production) for reproduction was determined using non-linear regression analysis in SYSTAT (version 13.0) (EC, 2005a). As soil concentrations were not determined for the soil toxicity test, only nominal test concentrations were used.

Measured tissue and nominal soil concentrations were used for all bioaccumulation calculations. Analysis of variance (ANOVA, $\alpha = 0.05$), followed by the Fisher's LSD test, were used to evaluate for significant differences between sampling days for tissue-chemical concentrations (uptake and elimination phases, separately); these tests of significance were also used to determine whether steady-state had been attained during the uptake phase. All mean values presented are accompanied by the standard error, unless otherwise indicated.

The soil samples, although collected, were not analyzed; samples were extracted, and measurements began, but then with storage, were lost and could not be found in order to complete the analysis. Therefore, a soil degradation rate (0.17 ± 0.011 d^{-1}, $r^2 = 0.95$) was used from Jager et al. (2000), of which soil losses from 50 and 100 mg phenanthrene kg^{-1} dry artificial soil was evaluated as part of an earthworm bioaccumulation study.

For the uptake phase, uptake and elimination rate constants were determined using a first-order two-compartment model, taking into account chemical degradation (i.e., 0.17 d^{-1}) (Landrum, 1989)

$$dC_b/dt = (k_u \times C_s(0) \times e^{-k_0 \times t}) - (k_e \times C_b) \qquad \text{(Eq. 4.1)}$$

where C_b represents the concentration (pg mite^{-1}) of chemical in mite tissue, t represents the exposure time (d), $C_s(0)$ represents the soil concentration the initial contaminant concentration in soil (mg kg^{-1}) (i.e., using nominal starting concentrations), k_u represents the uptake rate constant

(pg mite^{-1} d^{-1}), k_e is the elimination rate constant (d^{-1}), and k_0 is the chemical degradation rate (i.e., 0.17 d^{-1}) in artificial soil. When solved (integrated), the equation becomes

$$C_b(t) = [(k_u \times C_s(0))/(k_e - k_0)] \times [e^{-k0 \times t} - e^{-ke \times t}] \quad \text{(Eq. 4.2)}$$

where $C_b(t)$ represents the concentration (pg mite^{-1}) of chemical in mite tissue at time t (d).

For the elimination phase, the mite data were fit to the following one-compartment, first-order kinetic model, where the uptake rate constant is equivalent to zero

$$dC_b/dt = -k_e \times C_b \quad \text{(Eq. 4.3)}$$

which when solved, becomes

$$C_b(t) = C_b(0) \times e^{-k_d \times t} \quad \text{(Eq. 4.4)}$$

where $C_b(0)$ is the chemical concentration (pg mite^{-1}) within the mite tissue at the start of the elimination phase, and k_e is now referred to as k_d, the depuration rate constant (d^{-1}) (so as to distinguish it from the elimination rate constant determined as part of the accumulation model (Eq. 4.2)).

The depuration half-life ($t_{½w}$) (d) of the chemicals in the mites was then calculated as

$$t_{½w} = \ln(2)/k_d \quad \text{(Eq. 4.5)}$$

The kinetic bioaccumulation factor (BAF_k) (kg soil pg^{-1} mite^{-1}) was calculated as follows, using the uptake and elimination rate constants derived from Eq. 4.2

$$BAF_k = k_u/k_e \quad \text{(Eq. 4.6)}$$

The BAF_k was also calculated, correcting for the estimated lipid content (%) in mites [i.e., 13%, based on estimates provided by Convey (1992)], and soil organic carbon content (%)

$$BAF_{kc} = (k_u/\text{lipid content})/(k_e/\text{soil organic carbon content}) \quad \text{(Eq. 4.7)}$$

The biota-to-soil accumulation factor (BSAF) (kg soil pg^{-1} $mite^{-1}$) could not be determined based on chemical measurements in soil; however, nominal soil concentrations were derived over time, based on the degradation rate of phenanthrene in artificial soil presented by Jager et al. (2000) (i.e., 0.17 d^{-1}). As such, an estimated BSAF was derived using chemical measurements in tissue (pg $mite^{-1}$) and nominal concentrations in soil over time (mg kg^{-1})

$$BSAF = C_b(t)/C_s(t)) \quad \text{(Eq. 4.8)}$$

The BSAFs were also determined correcting for the estimated lipid content (%) in mites, and soil organic carbon (%) content

$$BSAF_c = (C_b(t)/\text{lipid content})/(C_s(t)/\text{organic carbon content}) \quad \text{(Eq. 4.9)}$$

4.5. Results

4.5.1. Soil toxicity test

The test concentrations were sufficient to capture both lethal and sublethal effects (Fig. 4.1), which were used to determine the effective concentrations at which to assess phenanthrene's bioaccumulation potential in the *O. nitens*. Overall, adult survival was acceptable in the control treatment (85 ± 15%), and the calculated LC50, using the Spearman-Kärber method of analysis was 388 mg kg^{-1} (95% CL: 330 - 446). The mean juvenile production was quite low (11 ± 2.0 individuals); however, reproduction on the whole was sufficient to statistically determine the 25 and 50% effect concentrations (IC25 = 48 mg·kg^{-1}, 95% CL: 15 - 146; IC50 = 95 mg·kg^{-1}, 95% CL: 26 - 345) using a hormetic regression model.

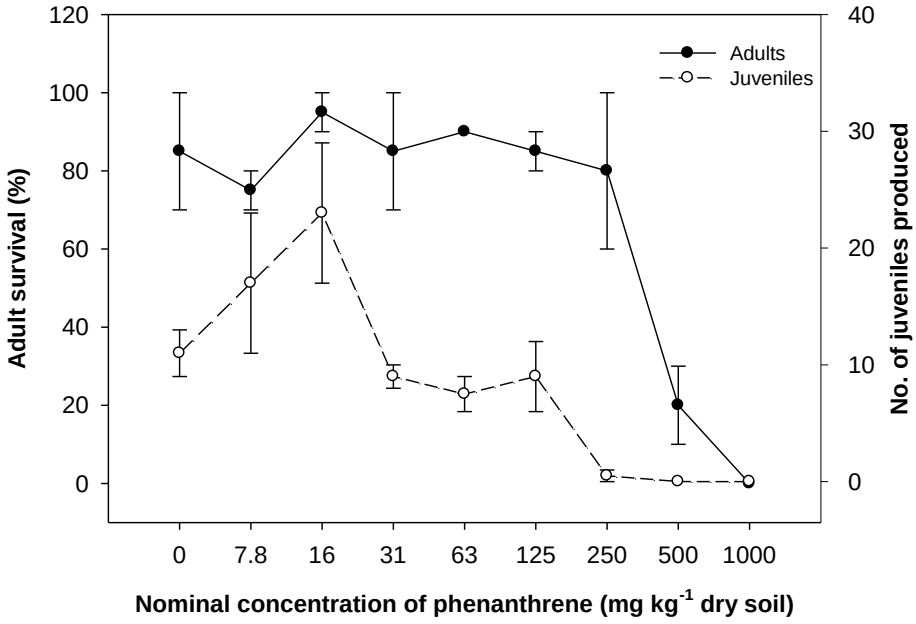

Fig. 4.1. Mean (± standard error) *Oppia nitens* adult survival (n = 3) and juvenile production after exposure to phenanthrene in artificial soil for 28 days.

4.5.2. Soil bioaccumulation test

There were no significant differences in adult survival across time intervals for the 100 (p = 0.14) and 200 (p = 0.20) mg kg^{-1} exposure studies; however, significant mortality, relative to the start of the test (i.e., day 0), was observed after 28 days in the 400 mg kg^{-1} exposure study (Fig. 4.2). There is no test validity established for the bioaccumulation test with *O. nitens*, however, using the OECD (2010) protocol as a guide, test validity would include an acceptable level of mortality at the end of the uptake phase (relative to the start of the test) of 10%. The test validity criteria was only achieved in the 200 mg kg^{-1} exposure (mean adult survival, 85 ± 8.7%), with decreased (although not significant (p = 0.20)) survival in the 100 mg kg^{-1} exposure (78 ± 10%), and a significant (p < 0.01) decrease in the 400 mg kg^{-1} exposure (43 ± 14%). Once the *O. nitens* were transferred to clean soil, there was no subsequent significant change in mortality across time intervals.

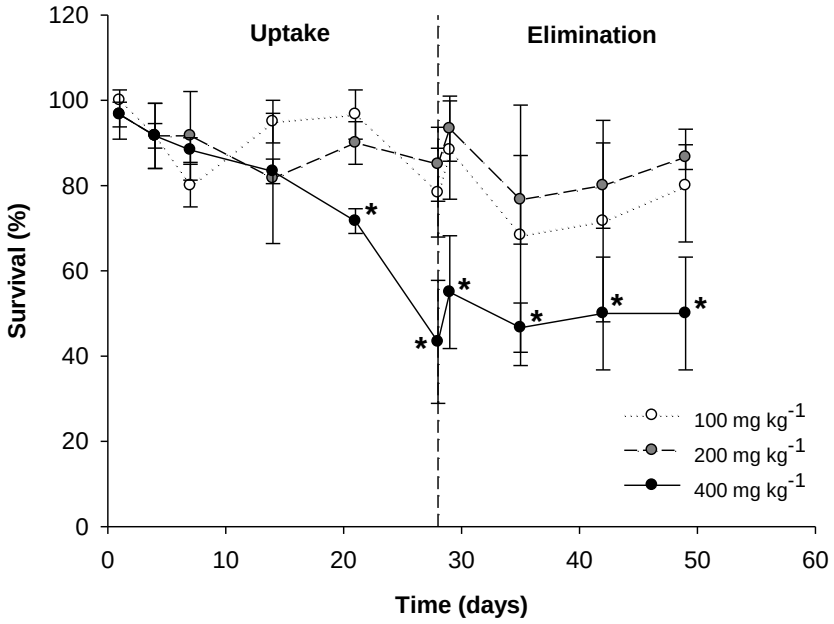

Fig. 4.2. Mean (± standard error) *Oppia nitens* adult survival (n = 3) upon exposure to phenanthrene in artificial soil for 28 days, followed by subsequent transfer to clean soil for an additional 21 days. Asterisks denote a significant difference ($p < 0.05$) relative to the start of the test (i.e., day 0).

The test results for the mite tissue analyses were varied, and once corrected, required the removal of negative values, and outliers in some instances (refer to Appendix B for raw data, where outliers and negative values are indicated). The results of the modeling were varied (refer to (Table 4.4) for kinetic rates, and Appendix B for the model graphs) in that *r*-square values were consistently low for the modeling of the elimination phase. This may have been due to measured values being close to the detection limit of the equipment, and/or the fact that significant mortality was evident (e.g., in the 400 mg kg^{-1} treatment).

Table 4.4. Rate constants for loss of chemical from artificial soil, and accumulation and elimination of phenanthrene by *Oppia nitens*.

Parameter	100 mg kg^{-1}	200 mg kg^{-1}	400 mg kg^{-1}
Loss from soil[†]			
k_0 (d^{-1})	0.17 ± 0.011	0.17 ± 0.011	0.17 ± 0.011
$t_{½s}$ (d)	4.1	4.1	4.1
Elimination phase			
r^2	0.012	0.065	0.25
k_d (d^{-1})	0.0051 ± 0.0074	0.018 ± 0.0085	0.036 ± 0.12
$t_{½w}$ (d)	139	39	19
Uptake phase			
r^2	0.58	0.37	0.87
k_u (pg mite^{-1} d^{-1})	0.0075 ± 0.0046	0.0077 ± 0.0055	0.0021 ± 0.00091
k_e (d^{-1})	0.15 ± 0.044	0.19 ± 0.066	0.076 ± 0.024
BAF_k[‡]	0.054	0.043	0.026
BAF_{kc}[§]	0.022	0.018	0.011
BSAF[¶]	1979 ± 574	1041 ± 781	1942 ± 162
$BSAF_c$[#]	805 ± 233	423 ± 318	790 ± 66

[†]Soil concentrations were not measured; therefore, the parameter reported by Jager et al. (2000), that pooled estimates from 50 and 100 mg kg^{-1} of phenanthrene in artificial soil.
[‡]BAF_k non-normalized bioaccumulation factor (calculated using the uptake (k_u) and elimination (k_e) kinetics from the uptake phase)
[§]BAF_{kc} normalized for lipid (13%) and soil organic carbon content (5.5%)
[¶]BSAF biota-to-soil accumulation factor; non-normalized (calculated using the d-28 measured tissue and calculated nominal soil concentrations from the uptake phase)
[#]$BSAF_c$ normalized for lipid (13%) and soil organic carbon content (5.5%)

The mean accumulation and depuration of phenanthrene for each exposure concentration are presented in Fig. 4.3. For the 100 and 200 mg kg^{-1} exposure treatments, there were no significant changes (100 mg kg^{-1} exposure: $p = 0.16$; 200 mg kg^{-1} exposure: $p = 0.12$) in tissue concentration evident; there were also no significant changes (100 mg kg^{-1} exposure: $p = 0.51$; 200 mg kg^{-1} exposure: $p = 0.16$) in tissue concentrations throughout the elimination phase. For the 400 mg kg^{-1} exposure, there was no significant change in tissue concentrations until day 21 to 28 ($p < 0.010$). For the elimination phase, there was a significant decrease ($p < 0.010$) in tissue concentrations between days 28 and 29; however, no significant changes ($p > 0.18$) in tissue concentrations occurred thereafter across time. In comparison to each treatment, greater accumulation was evident with each increasing treatment concentration, with the exception of the d-28 200 mg kg^{-1} mean (30 ± 22 pg mite^{-1}), which decreased to an amount equivalent to the d-28 100 tissue concentrations (28 ± 8.4 pg mite^{-1}) (Fig. 4.3). With regards to elimination, the concentration of phenanthrene in tissue decreased over time, such that regardless of starting concentration, tissue concentrations were relatively consistent after 35 days (Fig. 4.3).

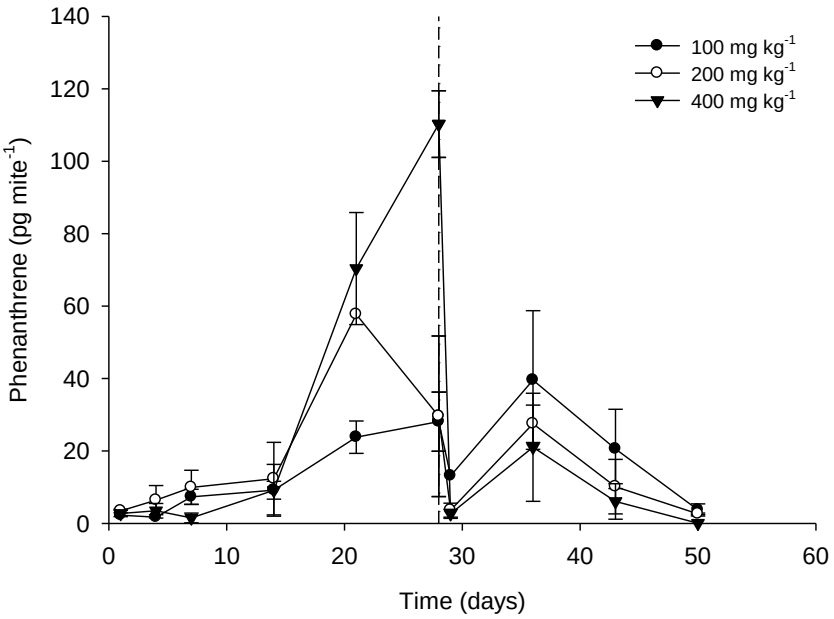

Fig. 4.3. Mean (± standard error) (n = 3) concentration of phenanthrene in *Oppia nitens* (pg per mite) upon exposure to nominal concentrations of 100, 200, and 400 mg kg^{-1} artificial soil for 28 days (referred to as the uptake phase), and subsequent transfer to clean soil (referred to as the elimination phase).

The depuration and uptake parameter estimates for the uptake and elimination phases associated with each exposure are presented in Table 4.4. The calculated d-28 BSAFs (non-normalized BSAF, and $BSAF_c$ normalized to lipid and soil organic carbon content) are also presented in Table 4.4; however, as they were estimated based on calculated nominal soil concentrations, they likely over-estimate the actual phenanthrene accumulation experienced by the mites. The mean $BSAF_c$ (corrected for lipid and soil organic carbon content) for each sampling date was also determined (Fig. 4.4), and there was a significant rise ($p < 0.010$) after 21 days for the 100 mg kg^{-1} exposure; no significant difference ($p = 0.13$) across time for the 200 mg kg^{-1} exposure; and a significant rise ($p < 0.011$) in the $BSAF_c$ after 14 days for the 400 mg kg^{-1} exposure (Fig. 4.4).

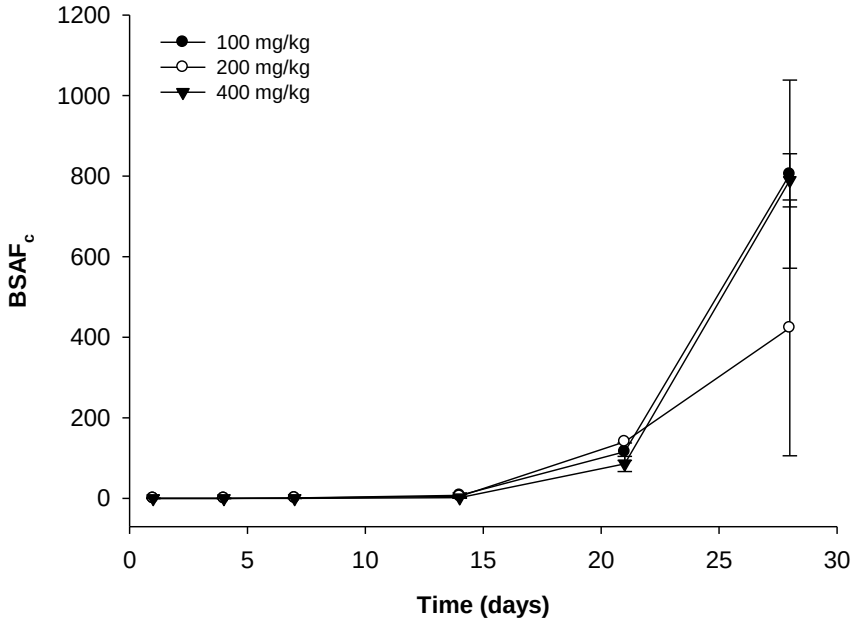

Fig. 4.4. Mean (± standard error) biota-to-soil accumulation factors ($BSAF_c$; normalized for lipid and soil organic carbon content) (n = 3) of phenanthrene for *Oppia nitens* exposed to phenanthrene in artificial soil at three concentrations: 100, 200 and 400 mg kg^{-1} dry soil.

4.6. Discussion

Phenanthrene was toxic to *O. nitens* at the tested concentrations, such that effects on adult survival and juvenile production were clearly discernible. Interestingly, a hormetic response was observed for reproduction, in that greater juvenile production was observed at the lowest test concentrations; this phenomenon also occurred when *O. nitens* were exposed to benzo[a]pyrene (Owojori and Siciliano, 2012), but also when evaluating for sublethal effects of phenanthrene on the enchytraeid, *Enchytraeus albidus* in LUFA 2.2 soil (Amorim et al., 2011), as well as the isopod, *Oniscus asellus*, when exposed to phenanthrene via food (van Brummelen et al., 1996a). Such stimulatory effects, at low PAH concentrations may in part be explained by the interference of PAH compounds with hormonal regulation (van Brummelen et al., 1996a), as both the metabolism of the PAHs and steroids involve the same P450 mixed-oxygenase systems (Goksøyr and Förlin, 1992). PAHs have been structurally compared to hormonal regulating steroids (e.g., ecdysteroids, estradiols), such that once metabolized, they may directly interfere and interact with hormone receptors involved in reproduction (Waxman, 1988; Hall and Oris, 1991). As

contaminant concentrations increase, PAHs may indirectly interfere with steroid metabolism through burdening detoxification mechanisms, or through resource allocation from reproduction to the detoxification and repair systems (Mazurová et al., 2008).

It was hypothesized that the oribatid mites might confer a greater degree of sensitivity to PAHs through pro-longed exposure due to their slow dispersal capacity, lowered metabolism, and preferred habitat of organic horizons, within which PAHs are mainly found (Cortet et al., 2006). However, in comparison to other soil invertebrates, *O. nitens* was as sensitive as the collembolan *Folsomia candida* in artificial soil, and the enchytraeid, *E. crypticus*, albeit in standard LUFA 2.2 natural soil, for the same test durations (i.e., 28 days) (Table 4.5). In comparison to a 14-d test in artificial soil using another mite species, the predatory *Hypoaspis aculeifer*, effects on *O. nitens* adult survival and reproduction were quite similar (Owojori et al., 2014). A comparison of lethal and sublethal responses of different soil invertebrates to phenanthrene has been presented in Table 4.5. However, it is difficult to generalize sensitivity across organisms, as the toxicity varies within and across species, and is likely soil-dependent. The variation in toxicity stresses the relevance and importance of using a test battery (i.e., multiple species, representative of different groups) in the assessment of contaminants in soil (Sverdrup et al., 2006). Although limited data are available in the literature, relative to other PAHs, *O. nitens* was more sensitive to phenanthrene than benzo[a]pyrene (LC50 and IC50 >1600 mg kg^{-1}) when exposed in artificial soil (Owojori and Siciliano, 2012). An additional study on the avoidance behaviour of phenanthrene to *O. nitens* in artificial soil (Owojori et al., 2011) yielded an EC50 (83 mg kg^{-1} (95% CL: 56 - 129)) quite similar to the reproductive effect levels observed herein.

Table 4.5. Comparison of LC50 and EC50 values (mg kg^{-1}) for phenanthrene derived from soil toxicity tests using different groups of soil invertebrates.

Species	Soil	Duration	LC50 (mg kg^{-1})	EC50 (mg kg^{-1})	Reference
Collembolans					
Folsomia candida	Artificial	28 d	380[#]	175 (148 - 192)	Crouau et al., 1999
Folsomia fimetaria	Natural[†]	21 d	41(38 - 45)	30 (23 - 36)	Sverdrup et al., 2001
Earthworms					
Eisenia fetida	Natural[‡]	14 d	41	-	Wu et al., 2011
Enchytraeids					
Enchytraeus crypticus	LUFA 2.2[§]	28 d	376	100	Droge et al., 2006
	LUFA 2.2	21 d	>400	145 (113 - 177)	Castro-Ferreira et al., 2012
	Natural[†]	21 d	>2000	87 (61 - 100)	Sverdrup et al., 2002
Enchytraeus albidus	LUFA 2.2	21 d	135 (84 - 216)	33 (26 - 44)	Amorim et al., 2011
Isopods					
Oniscus asellus	None[¶]	16 wks	>706	>706	van Brummelen et al., 1996
Porcellio scaber	None[¶]	47 wks	>706	>706	van Brummelen et al., 1996
Mites					
Oppia nitens	Artificial	28-d	388 (330 - 446)	95 (26 - 345)	(this study)
Hypoaspis aculeifer	Artificial	14 d	684 (405 – 1156)	49 (20 – 77)	Owojori et al., 2014
Snails					
Helix aspersa	Natural[†]	28 d	>2800	>2800[††]	Sverdrup et al., 2006

[†] Natural sandy loam soil (62% sand, 22% silt, 13% clay, 1.6% organic carbon)
[‡]Natural sandy loam soil (15% sand, 46% silt, 22% clay, 3.5% organic matter)
[§]Natural standard soil from Speyer, Germany (77% sand, 17% silt, 6% clay, 2.5% organic carbon, 4.4% organic matter)
[¶]Dietary exposure only
[#]Significant mortality occurred at this concentration; although LC50 was not determined, it likely fell between 220 and 380 μg g^{-1}
[††]EC10 for adult growth

Exposure studies using other soil invertebrate species demonstrate the ability for accumulation of PAHs (phenanthrene included), but with low body residue amounts, indicative of the ability for biotransformation and subsequent elimination, with little tendency for biomagnification (Jager et al., 2000; van Brummelen et al., 1996a; van Brummelen et al., 1996b). Indeed, *O. nitens* also accumulated phenanthrene, and proceeded to the eliminate the chemical once transferred to clean soil. Throughout the exposure duration (i.e., uptake phase), steady-state was not achieved. The uptake phase was characterized by a gradual accumulation of

the phenanthrene for approximately 14 days, followed by a rapid increase thereafter. This may be reflective of multiple routes of exposure, for example, the smooth and shiny cuticular surface exhibited by some oribatids (including *O. nitens*) is characteristic of a cerotegumental lipid layer (Raspotnig and Leis, 2009), thus allowing for dermal partitioning and the potential gradual accumulation within tissue measurements. However, thereafter, accumulation may then be a combination of both dermal and dietary uptake. Interestingly, although the phenanthrene was eliminated by the *O. nitens*, tissue residues at the end of the elimination phase (i.e., day 50; Fig. 4.3) were similar to those at the beginning of the uptake phase (e.g., days 1 to 7; Fig. 4.3), in that the phenanthrene was not completely eliminated, but possibly retained in the outer dermal lipid layer. Similar observations have been observed upon the exposure of the springtail *Folsomia candida* to naphthalene, whereby cuticular waxy layers were demonstrated to be a significant sorption phase within the first hours of exposure (Schmidt et al., 2013). This phenomenon may also contribute to increased sensitivity in immature stages, in part due to increased surface to volume ratio (i.e., larger for smaller immature mites) (Heckmann et al., 2005), and the rapid deposition of the cerotegument after moulting (as cited in Raspotnig and Leis, 2009). This requires further investigation, as the final exposure and susceptibility would be stage-specific, dependent on the time of exposure within the life stage, and when moulting occurs.

The degree to which the phenanthrene was accumulated varied with exposure treatment, with increased accumulation observed with increasing exposure concentration (Fig. 4.3). A peak in accumulation was evident on d-21 for the 200 mg kg^{-1} treatment (Fig. 4.3), however, this was not evident within the 400 mg kg^{-1} treatment (possibly in part due to adult mortality). Peak accumulation curves have also been observed in bioaccumulation studies of phenanthrene in other terrestrial invertebrates (Ma et al., 1995; Jager et al., 2000), attributable to either lipid loss, biotransformation (i.e., induced metabolism) or changes in bioavailability in the soil (Jager et al., 2000). As lipids were not measured, it is difficult to ascertain whether this would be a contributing factor. Biotransformation as a contributing factor is unlikely as metabolic induction would likely occur at a greater rate after exposure (i.e., within the elimination phase), when enzymes are optimally induced (Jager et al., 2000); however, the depuration (elimination) rate within the uptake phase was greater than that determined in the elimination phase for the 200 and 400 mg kg^{-1} treatments. As soil concentrations could not be measured, it is difficult to determine

whether changes in bioavailability (soil concentrations) were significant over time. Significant losses of phenanthrene have been observed in artificial soil over time (Jager et al., 2000; Hofman et al., 2008; Vlčková and Hofman, 2012). Hofman et al. (2008) described a biphasic decay curve comprising an initial short period of rapid loss due to microbial degradation, followed by a slow loss limited by mass transfer (i.e., desorption or dissolution from soil). This corresponds with Jager et al (2000), who theorized that the observed peak response for PAHs (phenanthrene included) was likely a combination of biodegradation (such that soil pore water concentrations are depleted at a faster rate by the soil microbial population, who are also in competition for the available chemical pool) and limited mass transfer. In contrast, Vlčková and Hofman (2012) propose that the initial loss of chemical is a result of volatilization before soil-contaminant processes come into effect, followed by microbial degradation (and then mass transfer limitations).

The calculated bioaccumulation factors (i.e., BAF_k and BAF_{kc}) for the *O. nitens* decreased slightly with increasing exposure concentration, but are relatively low compared to other species. A note in caution is required for comparative purposes, as soil concentrations were not measured, these values were derived based on nominal concentrations. The soil organic carbon (OC) and lipid-normalized kinetic-based bioaccumulation factors (i.e., BAF_{kc}, (kg OC kg $lipid^{-1}$ d) derived for some species include: (i) *Eisenia andrei*: 7.3 (21-d exposure in artificial soil (Jager et al., 2000)); *E. fetida*: ~1.5 falling to ~0.5 with increased aging of phenanthrene in artificial soil (10-d exposure (Vlčková and Hofman, 2012)); (iii) *Lumbricus rubellus* 0.024 g dry soil g fresh worm $weight^{-1}$ in sandy soil (28-d exposure (Ma et al., 1995); (iii) *E. albidus*: 8.3 (21-d exposure in artificial soil (Amorim et al., 2011)) and <2.5 (8-d exposure in artificial soils of varying organic carbon content (Hofman et al., 2008)). Given the analytical data derived from this study, *O. nitens* accumulated phenanthrene upon exposure to the chemical in soil, but also demonstrated the capacity to metabolize and eliminate the phenanthrene, similar to other soil invertebrate species (Jager et al., 2000; van Brummelen et al., 1996a; van Brummelen et al., 1996b). However, although likely limited, the potential for trophic transfer and biomagnification remains uncertain as residual levels of phenanthrene were evident towards the end of the elimination phase, possibly due to cuticular sorption processes.

High replicate variability was also observed throughout this study, regardless of initial exposure concentration. Other researchers have attributed high variability within bioaccumulation studies to heterogeneity of exposure in soil (e.g., Amorim et al., 2011); however, it is more likely that the analytical variability experienced herein was related to the number of mites used per replicate, and within the 400 mg kg^{-1} treatment, confounded by adult mortality. There was no significant mortality throughout the study (uptake and elimination phases) for the 100 ($p = 0.14$) or 200 ($p = 0.20$) mg kg^{-1} exposures; however, significant mortality ($p < 0.01$) occurred in the 400 mg kg^{-1} treatment after 21 days of exposure such that adult survival was only 43 ± 14%. However, once transferred to clean soil, there was no further significant ($p \geq 0.10$) mortality across time. This indicates that the treatment level caused a significant toxicological affect on survival, which is corroborated by the 28-d LC50 (388 mg kg^{-1} (95% CL: 330 - 466 mg/kg)). The higher concentration was selected to ensure the detection of phenanthrene in the mite tissue, however, given the level of mortality observed (Fig. 4.2), the decreased pool of organisms available for analysis likely confounded the accurate chemical analysis of the tissue. Twenty organisms per replicate (with three replicates per time point) were exposed to the phenanthrene-contaminated soils, and it could be that a greater pool of mites are required to accurately measure PAH concentrations. A study conducted by Owojori and Siciliano (2012) used 10 adult *O. nitens* per replicate to evaluate the effect of benzo[a]pyrene on survival and reproduction, including accumulation of the chemical in surviving adults at the end of the test. However, after 35 days, only trace values (below the detection limit) were obtained, irrespective of the starting test concentrations, which ranged from 50 to 1600 mg kg^{-1}. As a result, future efforts should be directed at examining the minimum pool of organisms required for the accurate analysis of mites when exposed to hydrocarbons in soil. Research demonstrates that oribatid mites are sensitive to hydrocarbon pollution in soil (Parmelee et al., 1993; Lebrun and van Straalen, 1995; Erstfeld and Snow-Ashbrook, 1999; Blakely et al., 2002), some of which may encompass various PAHs. Polycyclic aromatic hydrocarbons are likely to partition within soil organic components, particularly within soil horizons rich in organic matter (Jager et al., 2000; Krauss et al., 2000; Zhang et al., 2006); it is also within these horizons that oribatid mites are typically the most abundant microarthropod. Therefore, further tests are warranted to validate the observed trends, and confirm the bioaccumulation factors (i.e., BAFs and BSAFs) in order to determine the bioaccumulative nature and risk of PAHs to oribatid mites in general.

5. EVALUATION OF A NEW BATTERY OF TOXICITY TESTS FOR BOREAL FOREST SOILS: ASSESSMENT OF THE IMPACT OF HYDROCARBONS AND SALTS[2]

5.1. Preface

The initial focus of research encompassed the development of a new oribatid mite toxicity test. However, a comparison of the sensitivity of *O. nitens* to that of other boreal species was warranted. As a result, toxicity studies, using field-collected reference and contaminated (hydrocarbon- and salt-impacted) soils, were completed using an expanded suite of boreal species encompassing springtails (Collembola), earthworms and boreal tree and understory plants. The sensitivity of the boreal species was then compared to that of currently published standard soil toxicity test species (earthworms, Collembola and agronomic plants) to determine whether the boreal species were more or less sensitive to the contamination.

The research involved a collaborative effort among Environment Canada and the Saskatchewan Research Council (SRC). Although Environment Canada directed the overall research, the boreal plant tests were conducted by M. Moody at SRC. J. Princz directed the boreal invertebrate research at Environment Canada, although technical staff completed some of the testing (e.g., earthworm and Collembola). All invertebrate research was conducted at Environment Canada, including any tests using the published standard soil toxicity test species. J. Princz was responsible for the compilation, statistical analyses, and interpretation of results, particularly in the use of species-sensitivity distribution to describe the overall effects of the contaminated soils for the boreal and standard test species.

[2] Princz JI, Moody M, Fraser C, van der Vliet L, Lemieux H, Scroggins R, Siciliano SD. 2012. Evaluation of a new battery of toxicity tests for boreal forest soil: assessment of the impact of hydrocarbons and salts. Environmental Toxicology and Chemistry, 31(4): 766-777.

5.2. Abstract

The ability to assess the toxic potential of soil contamination within boreal regions is currently limited to test species representative of arable lands. This study evaluated the use of six boreal plant species (*Pinus banksiana*, *Picea glauca*, *Picea mariana*, *Populus tremuloides*, *Calamagrostis canadensis*, and *Solidago canadensis*) and four invertebrate species (*Dendrodrilus rubidus*, *Folsomia nivalis*, *Proisotoma minuta*, and *Oppia nitens*) and compared their performance to a suite of standard agronomic soil test species using site soils impacted by petroleum hydrocarbon (PHC) and salt contamination. To maintain horizon-specific differences, individual soil horizons were collected from impacted sites and relayered within the test vessels. Use of the boreal species was directly applicable to the assessment of the contaminated forest soils and, in the case of the hydrocarbon-impacted soil, demonstrated greater overall sensitivity (25th percentile of estimated species sensitivity distribution [ESSD25] = 5.6% contamination: 10,600 mg kg^{-1} fraction 3 [F3; equivalent hydrocarbon range of >C16 to C34] Of/Oh horizon, and 270 mg kg^{-1} F3 Ahg horizon) relative to the standard test species (ESSD25 = 23% contamination: 44,000 mg kg^{-1} F3 Of/Oh horizon, and 1,100 mg kg^{-1} F3 Ahg horizon). For salinity, there was no difference between boreal and standard species with a combined ESSD25 = 2.3%, equating to 0.24 and 0.25 dS m^{-1} for the Ah and Ck horizons. The unequal distribution of soil invertebrates within the layered test vessels can confound test results and the interpretation of the toxic potential of a site. The use of test species relevant to boreal ecozones strengthens the applicability of the data in support of realistic ecological risk assessments applicable to the boreal regions.

5.3. Introduction

Boreal regions account for approximately 57% of Canada's total land mass, of which boreal forests occupy approximately 35%, accounting for one third of the world's boreal forests (http:/www.atlas.nrcan.gc.ca/site/english/learningresources/theme_modules/borealforest/index.html). Resources within boreal regions significantly contribute to Canada's economy via energy (e.g., oil and gas), mining, and forestry industries. Although of economical value, these industries also inadvertently release pollutants; for example, petroleum hydrocarbon

contamination events, resulting from oil exploration, extraction, transport and processing, can result in surface and sub-surface soil contamination. It is estimated that >250,000 sites exist across the oil and gas sector that are contaminated by petroleum hydrocarbons (CCME, 2008); in addition, associated activities (e.g., oil extraction) may also potentially result in excess brine or salt contamination. These materials affect the soil ecosystem by directly eliciting a toxic effect and indirectly through the alteration of the soil habitat (e.g., increased osmotic potential, increased soil hydrophobicity, altered food-web dynamics) (Alexander, 2000; Blakely et al., 2002; Khasa et al., 2002).

The evaluation of contaminated site soils typically incorporates the use of a suite (or battery) of terrestrial organisms with a multitude of biological endpoints, and a range of sensitivities (Beirkens et al., 1998). The use of such an approach, using species representative of different guilds, allows for the consideration of various routes of uptake as well as varying toxicokinetic processes such as biotransformation or detoxification thus accounting for species- and endpoint-sensitivity. The ability to use a relevant test battery supports the development of realistic site-specific ecological risk assessments and remediation programs applicable to the affected sites, particularly if the test battery encompasses species native to the affected region or species that might be used as part of reclamation strategies.

Standardized terrestrial toxicity tests exist for a variety of soil-dwelling species [e.g., ISO, 1993; ISO, 1998; ISO, 1999). Within Canada, such standardized test methods have also been developed and validated for the assessment of contaminated site soils (EC, 2004; EC, 2005b; EC, 2007). However, the species used in the tests are better suited for arable regions, and their ecological relevance and applicability to Canadian non-temperate ecosystems such as boreal regions, is questionable (Römbke et al., 2006). As a result, research has been directed towards identifying and developing a new suite of terrestrial organisms suited to the assessment of contaminated sites in Canadian boreal regions (SRC, 2003; EC, 2010). For example, a series of proposed test plant species have been selected based on specific criteria relevant to their occurrence and prevalence within Canadian upland boreal forested sites, stress tolerance to environmental factors, as well as practical considerations such as seed availability, ability to germinate and grow under standardized test conditions, and ease of taxonomy (SRC, 2003). The

species include coniferous (*Pinus banksiana, Picea glauca* and *Picea mariana*) and deciduous (*Populus tremuloides*) tree plants, as well as understory species (e.g., *Calamagrostis canadensis* and *Solidago canadensis*). Not only are these species prevalent within boreal forests, and affected by industrial activities, they are also used in reclamation efforts (e.g., *P. glauca, P. tremuloides,* and *P. banksiana*) (Khasa et al., 2005).

Similarly, the need for boreal forest soil invertebrate species has been identified (Römbke et al., 2006), with recommendations based on origin (i.e., native), abundance, habitat, stress tolerance, but also practicality in acquiring and culturing species, in addition to definitive taxonomy. Based on these recommendations, efforts within this study were directed towards the collection, culturing, derivation and application of new test protocols for soil invertebrates collected from within Canadian boreal regions, or representative of species known to inhabit these areas; species of consideration included oribatid mites, Collembola and earthworms. Oribatid mites are most abundant within boreal forest soils (Crossley and Bohnsack, 1960; Walter, 1985), and have been used as indicators of soil quality, with observed susceptibility to petroleum hydrocarbon contamination (Blakely et al., 2002). Recently, an oribatid species, *Oppia nitens*, has been demonstrated to be an effective test species, applicable for use with boreal forest soils (Chapter 3). Collembola also comprise a significant portion of forest soil fauna, and are important in the maintenance and preservation of soil structure through their contributions to decomposition and nutrient cycling (Seastedt, 1984). Two particular species were collected from forest soils as potential test species: *Folsomia nivalis*, a parthenogenetic species, and *Proisotoma minuta*, a sexually-reproducing species. The earthworm species, *Dendrodrilus rubidus*, also present in boreal forest soils across Canada (Addison, 2009), was also collected.

To assess the impacts of contaminants on boreal forest ecosystems, this study evaluated the use of six ecologically-relevant boreal forest plant and four invertebrate species. The performance of the proposed boreal test species was compared to a suite of standard terrestrial test methods (EC, 2004; EC, 2005b; EC, 2007) using agronomic plants and soil invertebrates, and site soils impacted by petroleum hydrocarbon and salt contamination. In some instances, as in the case of a surface spill, soil horizons may remain unperturbed; therefore, this study included the collection of individual soil horizons from the field, and used a layered horizon

approach within the test vessels to mimic horizon depths observed in the field. This is in contrast to the traditional collection of soils, for example, from arable regions, where the soil is tilled, and horizons are disturbed resulting in a depth-based collection, and loss of horizon structure.

5.4. Materials and Methods

5.4.1. Site soils

Two contaminated site soils were collected from boreal regions within Alberta, with corresponding reference soils collected from adjacent unimpacted areas. The reference sites were selected to be as similar as possible to the contaminated soils, with the exception of contamination. All soils were collected in bulk, to a maximum depth of approximately 15-25 cm, with each soil horizon collected separately (refer to Table 5.1 for specific soil horizons). The soil was air-dried and sieved to either 6-mm (salt-impacted soil) or 8-mm (hydrocarbon-impacted soil, largely due to the fibric and humic material), homogenized and stored at room temperature (salt-impacted soil) or under refrigeration (4 ± 2°C) (hydrocarbon-impacted soil). Soil sub-samples were analyzed for general physical and chemical characteristics by Exova (Ottawa, ON) subsequent to homogenization, as well as throughout selected toxicity tests (Table 5.1).

The first site (SH07) was located in a treed bog within an active oil and natural gas field that had been impacted by a crude oil spill in 1989; some remedial efforts were evident at the site (e.g., straw used as an oil absorbent) (EcoDynamics Consulting Ltd., 2007). Soils were analyzed for petroleum hydrocarbon (PHC) content based on the Canada-Wide Standard (CWS) for PHCs in soil, which are regulated based on equivalent carbon ranges: Fraction 1 (F1) (C6 to C10), Fraction 2 (F2) (>C10 to C16), Fraction 3 (F3) (>C16 to C34) and Fraction 4 (F4) (>C34) (CCME, 2008). The upper horizon had greater PHCs (Table 5.1), with F2, F3 and F4 exceeding the CWS for PHCs Tier 1 remedial standards; F2 and F3 Tier 1 remedial standards were also exceeded in the lower horizon (CCME, 2008).

Table 5.1. Physical and chemical characteristics of the hydrocarbon and salt-impacted soils; the soils were collected by individual horizons. Two horizons (Of/Oh and Ahg) were collected from the SH07 reference and contaminated sites. Two horizons (Ah and C) were also collected from the PR07 reference site, but as there were no distinct horizons present at the contaminated site, a mixed horizon was collected.

	Hydrocarbon-impacted Site (SH07)				**Salt (Brine)-impacted Site (PR07)**		
Parameter	**Reference Site**		**Impacted Site**		**Reference Site**		**Impacted Site**
Horizon	Of/Oh	Ahg	Of/Oh	Ahg	Ah	Ck	Mixed
Site History	Undisturbed		crude oil spill (~1989)		Undisturbed		Orphaned gas well; continuous discharge of salt-laden mineral-rich groundwater
Forest Cover	Black spruce		75% of trees have been cut with some trees dead; 35% of site has no vegetation		Proximal aspen, white spruce forest, occasional black spruce wetlands, northern native grasslands		None
Understory	Mosses, reed grass, horsetail, bog Labrador tea		Mosses (haircap), red currant		Brome grass, rose, northern bedstraw, fireweed		None
Soil Type	Rego / Orthic Humic Gleysol		Rego / Orthic Humic Gleysol		Rego Dark Gray Chernozem		Mixed
Soil Texture	Peaty	Sandy-loam	Peaty	Sandy-loam	Sandy-loam	Sandy-loam	Loam
Electrical Conductivity ($dS\ m^{-1}$)	0.38	0.10	0.08	0.08	0.34	0.20	11
pH	3.9	4.3	4.6	4.7	7.1	7.7	8.4
Sodium Adsorption Ratio	0.88	1.3	2.3	2.6	1.2	1.2	65
Total Kjeldahl Nitrogen (%)	2.0	0.63	1.0	1.1	0.43	0.09	0.12
Total Organic Carbon (%)	35	11	52	15	6.3	1.5	1.2
Sodium (extractable) ($mg\ kg^{-1}$)	57	28	57	61	9.0	12	7,240
Calcium (extractable) ($mg\ kg^{-1}$)	462	570	388	810	3,380	2,400	2,560
Potassium (extractable)	53	81	31	72	430	203	209

	Hydrocarbon-impacted Site (SH07)				Salt (Brine)-impacted Site (PR07)		
Parameter	Reference Site		Impacted Site		Reference Site		Impacted Site
Horizon	Of/Oh	Ahg	Of/Oh	Ahg	Ah	Ck	Mixed
(mg kg^{-1})							
Phosphorous (extractable) (mg kg^{-1})	28	33	9.0	9.0	17	8.0	6.0
Fraction F1 (C6-C10) (mg kg^{-1}) †	<100	<20	<100	34	<20	<20	<20
Fraction 1-BTEX (C6–C10) (mg kg^{-1}) †	<100	<20	<100	33	<20	<20	<20
Fraction 2 (C10–C16) (mg kg^{-1}) †	<60	<20	22,600	754	<20	<20	<20
Fraction 3 (C16-C34) (mg kg^{-1}) †	409	271	190,000	4,970	55	<20	<20
Fraction 4 (C34-C50) (mg kg^{-1}) †	473	<20	41,000	1,600	39	<20	<20

†Petroleum hydrocarbon content was analyzed based on equivalent carbon ranges: Fraction 1 (F1) (C6 to C10), Fraction 2 (F2) (>C10 to C16), Fraction 3 (F3) (>C16 to C34) and Fraction 4 (F4) (>>C34) (CCME, 2008).

The second site (PR07) was associated with an orphan gas well that had been impacted by a continuous discharge of salt-laden, mineral-rich groundwater, despite past capping attempts. The contaminated soil was collected from around the abandoned gas well, and lacked a natural soil profile due to past disturbances by drilling and remediation efforts (EcoDynamics Consulting Ltd., 2007). Relative to the reference site, the contaminated soil had a high electrical conductivity (EC) and sodium adsorption ratio (SAR) (Table 5.1), which was several times higher than recommended Canadian interim soil remediation criteria for these parameters (i.e., EC of 2 to 4 dS/m and SAR of 5 to 12, depending on land use) (CCME, 2007).

5.4.2. Soil toxicity tests

Soil toxicity tests were performed by diluting the contaminated soil with the corresponding reference soil, to create a series of test concentrations. As distinct soil horizons

were collected at each site, the horizons were reassembled into the test vessels. For example, the upper Of/Oh horizon from the SH07 impacted site was diluted with the Of/Oh horizon from the SH07 reference site; the same procedure was applied to the lower horizons as well. For the PR07 soils, the upper (Ah horizon) and lower (Ck) reference soil horizons were each diluted with the same salt-impacted soil because of the perturbed soil profile. The horizons were reassembled using a 50% distribution of each horizon by volume within the test vessels, based on the depth of the horizons upon collection.

5.4.2.1. Plants

Plant tests were performed based on Environment Canada's standardized early seedling growth test method (EC, 2005b). Six boreal forest species were used: *Populus tremuloides* (trembling aspen), *Calamagrostis canadensis* (bluejoint reedgrass), *Picea mariana* (black spruce), *Picea glauca* (white spruce), *Pinus banksiana* (jack pine) and *Solidago canadensis* (Canada goldenrod). The plant seeds were either available commercially, or collected from wild populations (e.g., *P. tremuloides*), and required cold (i.e., 4 ± 2°C) stratification (14 days for *C. calamagrostis* and *P. banksiana*, and 21 days for *P. glauca*, *P. mariana*, and *S. canadensis*) in moist peat moss prior to use in testing; *P. tremuloides* did not require stratification, but was used from frozen seed stock. For comparison purposes, two standard test species (EC, 2005b) were also used: *Elymus lanceolatus* (northern wheatgrass) and *Trifolium pratense* (red clover). In brief, tests were performed in 1-L polypropylene test vessels, filled with 500-ml of the layered test soils. The number of seeds planted within test vessels varied by species and encompassed either five (*P. tremuloides*, *C. canadensis*, *S. canadensis*, *E. lanceolatus* and *T. pratense*) or 10 seeds (*P. mariana*, *P. glauca* and *P. banksiana*). Replication varied with species and concentration, with six to nine replicates used for the control, three to seven replicates used for the lowest four test concentrations, and three to five replicates for the remaining five test concentrations. Each test included a negative control treatment (e.g., formulated artificial soil) whereby plant emergence and growth were assessed against test validity criteria. For the standard plant species, test vessels were incubated at 24 ± 3°C in full spectrum lighting (300 ± 100 µmol/m^2·sec) for 16 h, and at 15 ± 3°C for 8 h of darkness; for comparative purposes, and to optimize plant growth in a reasonable length of time, the boreal forest plant test vessels were

incubated at 24 ± 3°C constant using the same full spectrum lighting regime. The relative air humidity was approximately 50 and 90% for the standard and boreal species, respectively; test vessels were watered with de-ionized water as needed (i.e., when the soil surface became dry). Test endpoints included mean seedling emergence, shoot and root length and shoot and root dry biomass. Test durations were species-dependent: 14 days for *T. pratense*, 21 days for *E. lanceolatus*, 28 days for *P. tremuloides* and *C. canadensis*, 35 days for *P. banksiana* and *S. canadensis*, and 42 days for *P. glauca* and *P. mariana*. The longer test durations used for the boreal forest species were selected based on the time required to attain sufficient biomass for the measurement endpoints.

5.4.2.2. Soil invertebrates

Soil invertebrate tests were performed based on Environment Canada's earthworm and collembolan soil toxicity test methods (EC, 2004; EC, 2007). As no prior standardized test method exists for oribatid mites, the methodology of Chapter 3 was used. The following forest soil invertebrates were used: the earthworm, *Dendrodrilus rubidus*; the Collembola, *Folsomia nivalis* and *Proisotoma minuta*; and the oribatid mite, *Oppia nitens*. The *Dd. rubidus* were collected from 49°69.17'N, 112°82.40'W (near Lethbridge, Alberta), and reared at 20 ± 3°C in a moistened artificial soil substrate (pH 7.0 ± 0.5) comprised of artificial and organic triple-mix soil, and peat; the earthworms were fed weekly using cooked oatmeal. The *F. nivalis* and *P. minuta* were collected from 53°67.61'N, 104°21.64'W (near Love, Saskatchewan), and reared at 20 ± 3°C in culture containers lined with an 8:1 plaster of Paris and charcoal substrate. The substrate was moistened, and baker's yeast added as a food source, weekly. The provenance and culture conditions for *Oppia nitens* were as described in Chapter 3. Similar to the plant testing, two standard soil invertebrate species (EC, 2004; EC, 2007) were also used: the earthworm, *Eisenia andrei*, and the springtail, *Folsomia candida*; there are currently no recommended standard test species for oribatid mites.

For earthworms, tests were performed in either 250- (*Dd. rubidus*) or 500-ml (*E. andrei*) glass test vessels, filled with 200 or 350 ml of the layered test soil, respectively. For *Dd. rubidus*, four sexually mature adults were added to each replicate test vessel; there were three

replicates per test concentration. The test vessels were incubated for 28 days, after which the adults were removed, and the test vessels incubated for another 28 days to allow cocoons to hatch. For *E. andrei*, two sexually mature adults were added to each replicate, and incubated for 35 days, after which the adults were removed, and the test vessels incubated for another 28 days; there were ten replicates per test concentration. All test vessels were incubated at 20 ± 3°C for 16 (*E. andrei*) or 12 h (*Dd. rubidus*) of light (>800 lux) and 8 or 12 h of dark, respectively. Test endpoints included 28- or 35-d mean adult survival, and 56- or 63-d mean juvenile production (measured as the total number of juveniles produced per replicate) and individual juvenile dry mass. At the end of the test, the horizons were separated, and the endpoints measured in each horizon.

Collembolan tests were performed in 125-ml glass test vessels, filled with 45 ml of the layered test soil. Age-synchronized individuals were used for testing, with the number of individuals varying by species: ten 10-12-d age-synchronized *F. candida* (total of 10 females) per replicate; five 16-18-d age-synchronized *F. nivalis* (total of 5 females) per replicate; and ten 10-12-d age-synchronized *P. minuta* (total of 5 females and 5 males) per replicate. Test vessels were incubated at 20 ± 3°C for 16 h of light (>800 lux) and 8 h of dark; there were three replicates per test concentration, except for the controls, which used five or six. Test durations were species-dependent: 21 days for *P. minuta* and *F. nivalis*, and 28 days for *F. candida*. Test endpoints included mean adult survival and juvenile production. Because of their quick mobility, endpoints were not tallied on a horizon-specific basis within the layered test vessel.

The oribatid mite test, using *O. nitens*, was performed according to Chapter 3. A 15-ml glass test vessel was filled with 7 ml of layered test soil, with ten mature adults added to each replicate. The test vessels were incubated at 20 ± 3°C for 16 h of light (>800 lux) and 8 h of dark; there were three replicates per test concentration. Test endpoints included 28-d mean adult survival and juvenile production. Similar to the earthworms, the endpoints were determined in each horizon of the layered test vessel.

5.4.3. Statistical analysis

Statistical analysis was performed according to the guidance outlined (EC, 2005a). A comparison between mean juvenile individual dry mass for the earthworms was assessed using analysis of variance, followed by pair-wise comparison analysis using the Fisher's least significant difference test. The EC50 (or 25) and LC50 (or 25) (i.e., concentration causing a 50 or 25% effect) for seedling emergence and adult lethality was determined using probit analysis; however, if model assumptions could not be met, the Spearman-Kärber method of analysis was used. The toxicity estimates were then compared using the Litchfield-Wilcoxon method (EC, 2005a). The IC50 (or 25) (i.e., concentration causing a 50 or 25% inhibition in growth or reproduction) for plant growth and reproduction was determined using non-linear regression analysis using SYSTAT (version 12.0) (EC, 2005a). However, if assumptions for normality and homogeneity of variance, assessed using Shapiro-Wilk's and Levene's tests respectively, could not be met, the data were reanalyzed using linear interpolation (Norberg-King, 1993), and the expanded confidence limits used in cases where there were less than seven replicates per treatment (EC, 2005a). Statistical significance between estimates was determined through a comparison of confidence intervals (EC, 2005a); toxicity estimates were significantly different when confidence limits did not overlap. Effect measures (i.e., LC25s and IC25s, expressed as a percent of diluted contaminated soils) were ranked according to sensitivity, based on guidance from Alberta Environment, Canada (Alberta Environment, 2007), and plotted as cumulative frequency distributions using a probability scale on the y-axis, and a logarithmic scale on the x-axis using SigmaPlot (version 9.0) to generate estimated species sensitivity distributions (ESSD). Three data sets were evaluated: the proposed boreal species; the standard species; and the boreal and standard species together. Linear regressions on the transformed data were used to generate the effect concentration (i.e., percent of diluted contaminated soil) corresponding to the 25^{th} percentile, expressed as the ESSD25, for each data set. The corresponding ESSD25 was then determined using actual measured contaminant levels. For the hydrocarbon-impacted soil, F3 was selected to represent hydrocarbon toxicity because it was present in excessively high concentrations, it exceeded Tier 1 remedial standards set by the CWS for PHCs in both soil horizons, and contained greater recalcitrant hydrocarbons (i.e., higher molecular weight) relative to F2. Soil conductivity was selected to represent contaminant levels in the salt-impacted soils.

5.5. Results

5.5.1. Species performance

Species performance was assessed by evaluating the degree of growth, survival or reproduction in the artificial (where possible) and reference site soils; artificial soil control replicates were not performed in tandem with the contaminated soil tests for the *Dd. rubidus, F. nivalis* and *O. nitens*. As a result, for comparative purposes, prior test data were used for *Dd. rubidus*, and data from Chapter 3 were used for *O. nitens*; there was no artificial soil testing conducted with *F. nivalis*. Plant test validity criteria consisted of mean seedling emergence and shoot and root length, and invertebrate test validity criteria included mean adult survival, juvenile production, and individual juvenile dry mass (earthworms only). Test validity for the standard test species was measured based on species performance in artificial soil (EC, 2004; EC, 2005; EC, 2007), and in each instance, the test validity criteria were met. As no test validity criteria are yet available for the proposed boreal test species, acceptable variation in species performance was evaluated in all three control soils, using the application of a coefficient of variation (CV) less than 30% (ISO, 1999).

For the boreal plants, seedling emergence was >70% in all control soils, with the exception of *C. canadensis* (69%) exposed to artificial soil, and *P. glauca* (17 – 53%) and *S. canadensis* (40 – 57%), regardless of soil type (Fig. 5.1). Although plant growth criteria established for the standard test species are species-specific, and cannot be applied to the boreal plants, an emergence of >70% is desired, irrespective of plant species (EC, 2005b). Shoot and root length varied between species and soil type (Fig. 5.1), with CVs <30% with the following exceptions: *P. glauca* root length in artificial (49%) and salt reference (38%) soils; and *S. canadensis* shoot (37%) and root (33%) length in artificial soil, shoot length (35%) in the hydrocarbon reference soil, and shoot (33%) and root (35%) length in the salt reference soil.

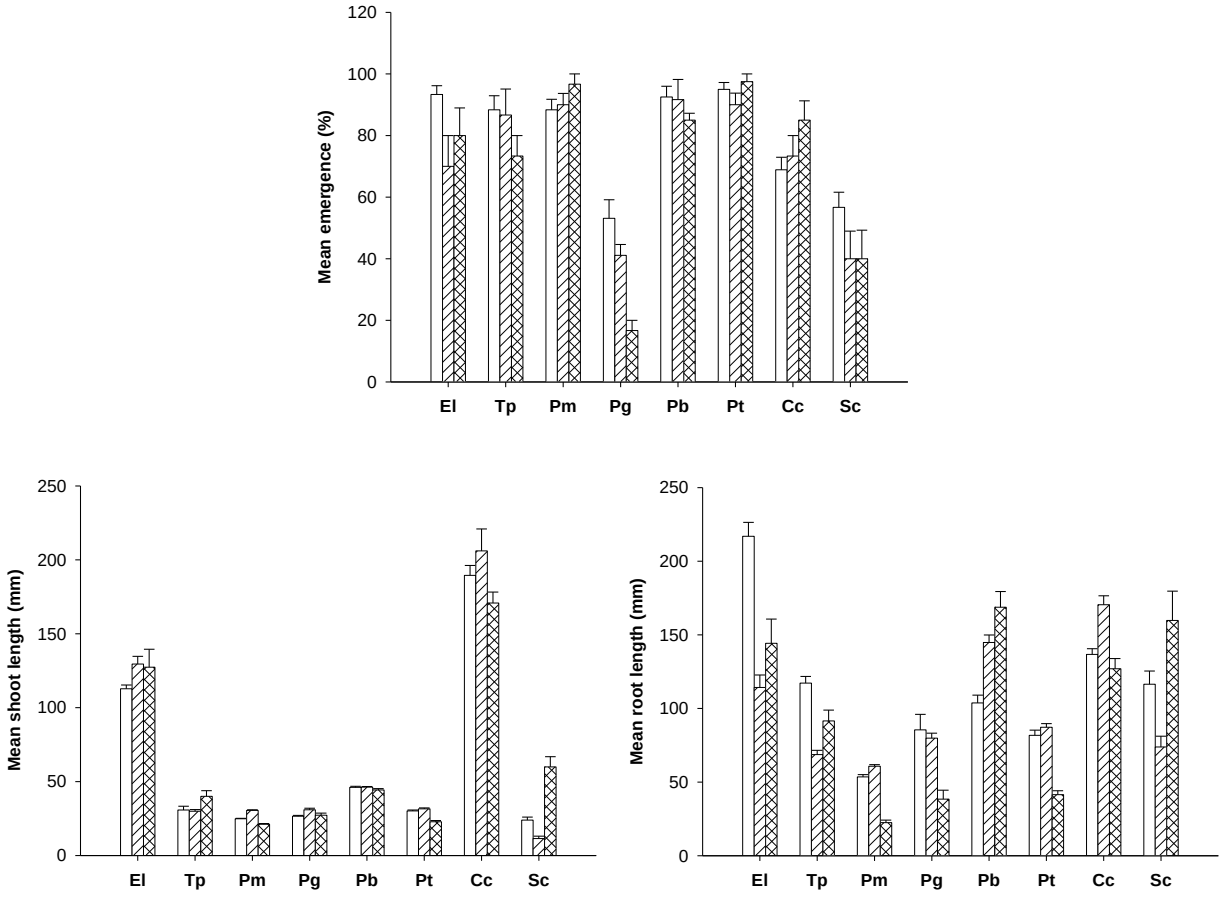

Fig. 5.1. Plant growth in control replicates of artificial (AS) (*n* = 12-18) (blank bars), hydrocarbon-impacted reference (*n* = 6-10) (hatched bars), and salt-impacted reference (*n* = 6-9) (cross-hatched bars) soils; data for the AS were pooled between tests. Error bars represent the standard error of the mean. Abbreviations are: El = *Elymus lanceolatus*, Tp = *Trifolium pratense*, Pm = *Picea mariana*, Pg = *Picea glauca*, Pb = *Pinus banksiana*, Pt = *Populus tremuloides*, Cc = *Calamagrostis canadensis* and Sc = *Solidago canadensis*.

For the boreal invertebrate species, adult survival was >70% in all control soils, with corresponding CVs <30%, with the exception of *F. nivalis*, which had low adult survival and high variation (CV = 151%) in the salt reference soil (Fig. 5.2). With the exception noted (i.e., *F. nivalis*), the adult survival test validity criteria for the standard test species (i.e., >90% survival for *E. andrei*, >70% for *F. candida* in a field soil) (EC, 2004; EC, 2007) was also met for the boreal species. In general, juvenile production was lower for the boreal collembolan species when compared to the standard test species; however, the mean juvenile production for *F. nivalis* and *P. minuta* was >100 juveniles thereby meeting the test validity criterion

established for the standard test species, *F. candida* (EC, 2007). Similarly, juvenile production for *Dd. rubidus* also met the test validity criterion established for *E. andrei* (i.e., >3 live juveniles/adult) (EC, 2004). In fact, for soils where reproduction was evident, the mean number of juveniles was greater for *Dd. rubidus* relative to *E. andrei*. There is no standard oribatid mite test method with which to compare the results from testing with *O. nitens*, however, reproduction was less than that observed for the other invertebrate species. The CVs for juvenile production were <30% for the boreal species, with the following exceptions: *F. nivalis* (54%) in the hydrocarbon reference soil, *P. minuta* (47 – 57%) regardless of soil type, and *O. nitens* (81%) in the artificial soil. Mean individual juvenile dry mass for the earthworms was similar between the two species, with corresponding CVs <30%; *Dd. rubidus* met the same test validity criterion established for *E. andrei* (i.e., >2.0 mg) (EC, 2004).

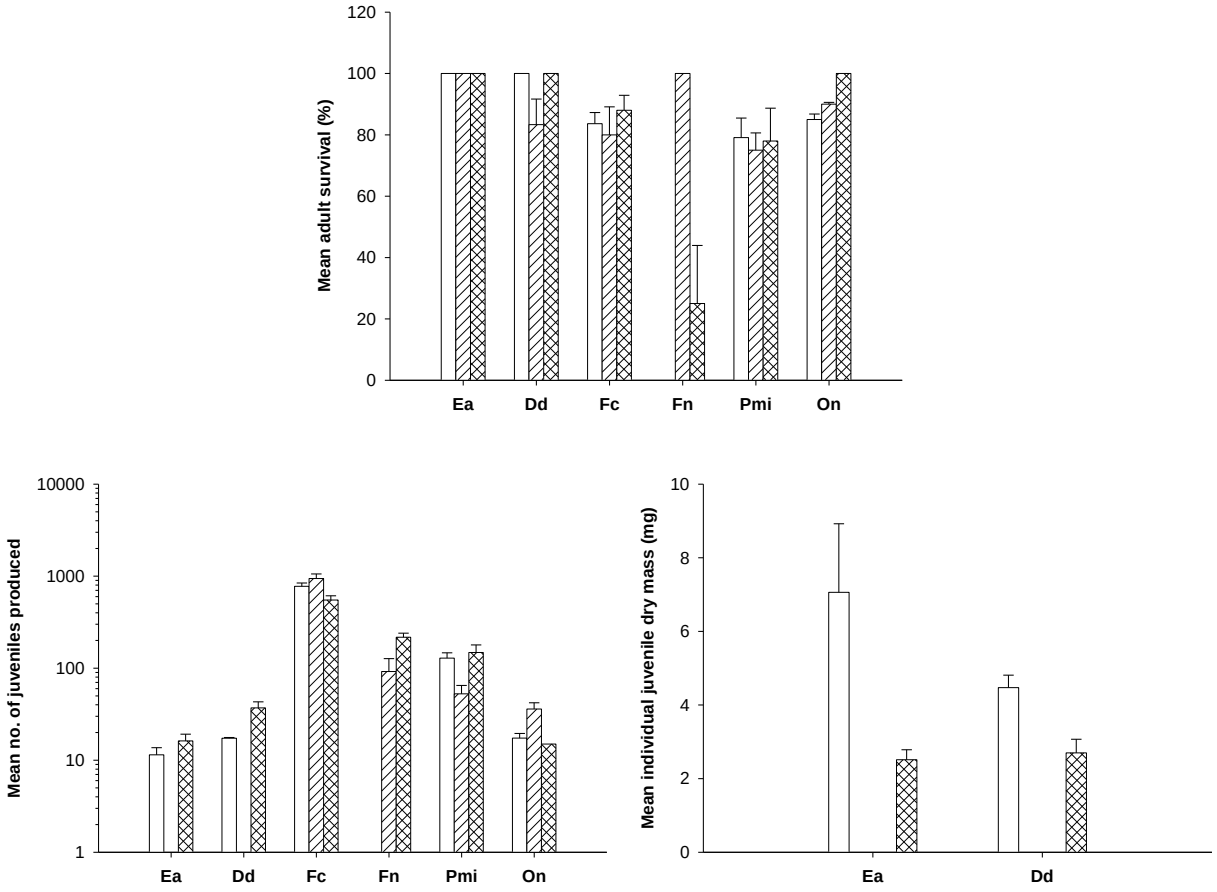

Fig. 5.2. Mean adult survival, mean juvenile production, and mean individual juvenile dry mass (earthworms only) in control replicates of artificial (AS) (blank bars), hydrocarbon-impacted reference (SH07) (hatched bars), and salt-impacted reference (PR07) (cross-hatched bars) soils; data for the AS were pooled between tests. Error bars represent the standard error of the mean. Abbreviations are: Ea = *Eisenia andrei* (*n* = 10-12), Dr = *Dendrodrilus rubidus* (*n* = 3), Fc = *Folsomia candida* (*n* = 5-11), Fn = *Folsomia nivalis* (*n* = 4), Pmi = *Proisotoma minuta* (*n* = 5-11), and On = *Oppia nitens* [*n*=3-5 (Chapter 3)].

5.5.2. Soil toxicity tests – species sensitivity

5.5.2.1. Hydrocarbon-impacted soils

Seedling emergence was not affected by exposure to the contaminated soil, and test results indicated a similarity in response (as measured by effects on plant growth) between the boreal and standard species. However, *C. canadensis* (all endpoints) and *P. tremuloides* (all

endpoints except shoot dry mass) were more sensitive to the hydrocarbon-impacted soils, regardless of endpoint, relative to all other plant species assessed (Fig. 5.3).

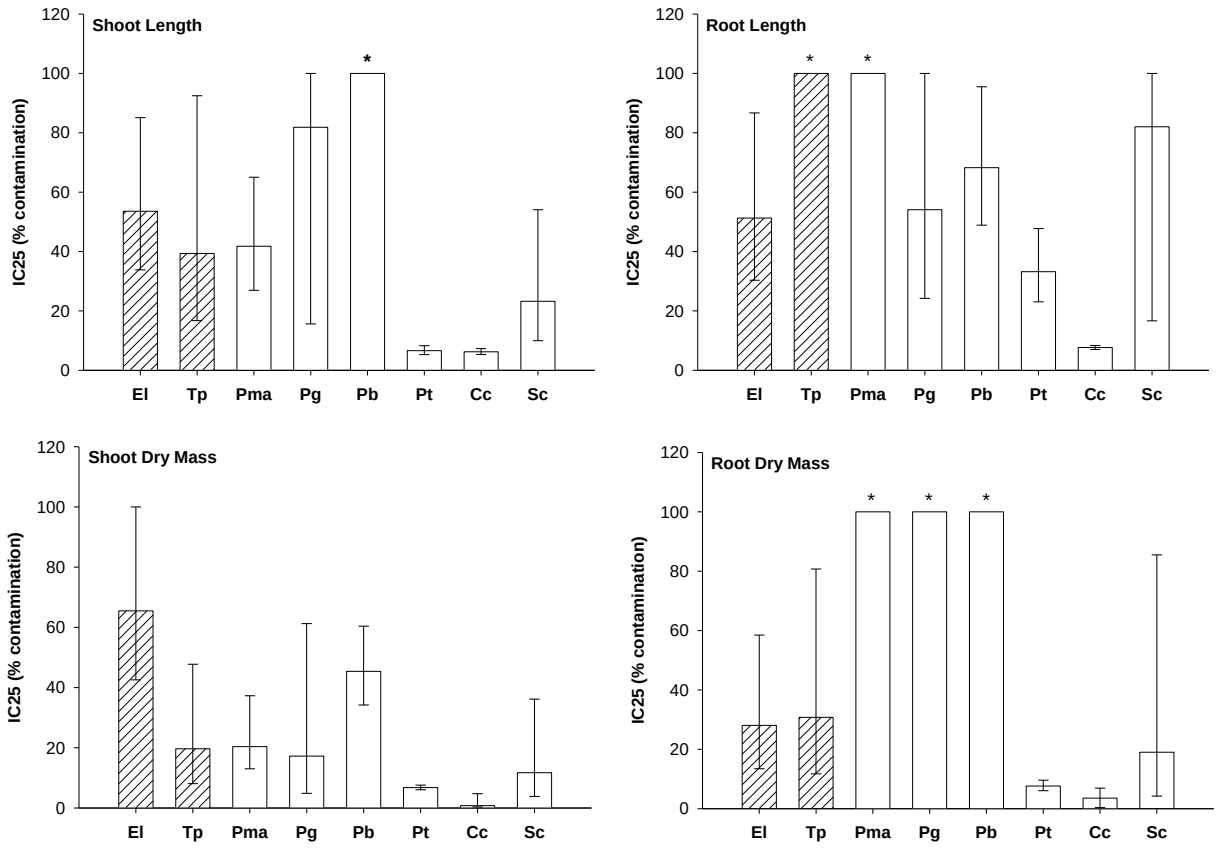

Fig. 5.3. The effect of hydrocarbon contamination on plant growth, measured by shoot and root length, and dry biomass. The y-axis represents the IC25, indicative of the concentration resulting in a 25% reduction in response relative to the control. Hatched bars are standard test species, and blank bars are the boreal forest test species. Error bars denote the upper and lower 95% confidence limits. Asterisks denote no effect at the highest concentration tested (100% contamination). Abbreviations are: El = *Elymus lanceolatus*, Tp = *Trifolium pratense*, Pm = *Picea mariana* (*n* = 5-6), Pg = *Picea glauca* (*n* = 5-9), Pb = *Pinus banksiana* (*n* = 5-6), Pt = *Populus tremuloides* (*n* = 5-8), Cc = *Calamagrostis canadensis* (*n* = 5-9) and Sc = *Solidago canadensis* (*n* = 5-6).

Adult survival of both earthworm species was unaffected by the contaminated soil (Fig. 5.4); however, there was no reproduction in either reference and contaminated soils (Fig. 5.4). Of the collembolan species, the boreal forest species were significantly more sensitive to the contaminated soil than the standard test species, regardless of test endpoint (Fig. 5.4). There is no comparative test species for the oribatid mite, *O. nitens*. Adult survival was unaffected by the

test soil, however, reproduction was affected at concentrations similar to those observed for the collembolan species (Fig. 5.4).

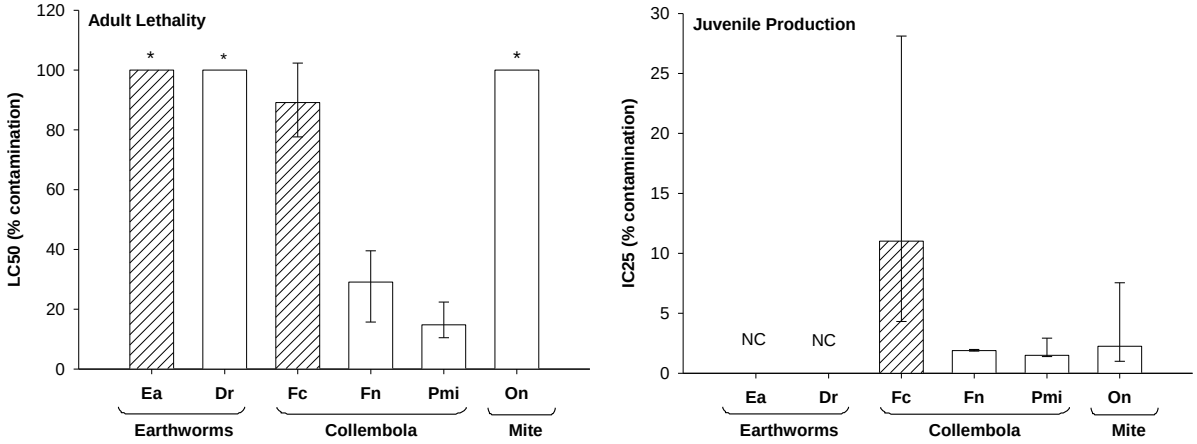

Fig. 5.4. The effect of hydrocarbon contamination on soil invertebrate reproduction. The y-axis represents the LC50 (lethality) or IC25 (reproduction), indicative of the concentration resulting in a 50 or 25% reduction in response relative to the control. Hatched bars are standard test species, and blank bars are the boreal forest test species. Error bars denote the upper and lower 95% confidence limits. Asterisks denote no effect at the highest concentration tested (100% contamination). Abbreviations are: Ea = *Eisenia andrei* (*n* = 10), Dr = *Dendrodrilus rubidus* (*n* = 3), Fc = *Folsomia candida* (*n* = 3), Fn = *Folsomia nivalis* (*n* = 3), Pmi = *Proisotoma minuta* (*n* = 3), On = *Oppia nitens* (*n* = 3), and NC = not calculable.

Estimated species sensitivity distributions (ESSDs) were generated to determine whether the boreal and standard test battery of species exhibited differences in their overall sensitivity to the contaminated soil. These distributions can be used to estimate a percentile effect level (e.g., 25th percentile or ESSD25) to support the derivation of site-specific remediation objectives for contaminated lands (Alberta Environment, 2007). In this example, Fraction 3 (>C16 to C34) was selected to represent hydrocarbon toxicity because it was present in high concentrations in both horizons, it exceeded Tier 1 remedial standards set by the CWS for PHCs in soil, and contained more recalcitrant hydrocarbons (i.e., higher molecular weight) relative to F2 (which also exceeded the Tier 1 remedial standards). With regards to test battery, the suite of boreal species was more sensitive to the hydrocarbon-impacted soils, relative to the suite of standard test species (Fig. 5.5). For example, the 25th percentile for the boreal species combined was 5.6% contamination, which equates to 10,682 mg kg^{-1} F3 for the Of/Oh horizon, and 265 mg kg^{-1} F3 for the Ahg horizon; the species and endpoints most at risk (i.e., falling below the 25th

percentile) included *C. canadensis* shoot and root dry mass, *F. nivalis* survival and reproduction, *P. minuta* reproduction and *O. nitens* reproduction. For the standard test species, the 25th percentile was 23% contamination, equating to 43,504 mg kg^{-1} F3 and 1,078 mg kg^{-1} F3 for the Of/Oh and Ahg horizons, respectively; the species and endpoints most at risk included *T. pratense* shoot dry mass and *F. candida* reproduction. When combined, the resultant 25th percentile was 7.4% and was driven by the sensitivity of the boreal species, rather than the standard test species; species and endpoints falling below this percentile included *C. canadensis* shoot length and shoot and root dry mass, *P. tremuloides* shoot length and dry mass, *F. nivalis* survival and reproduction, and *P. minuta* and *O. nitens* reproduction.

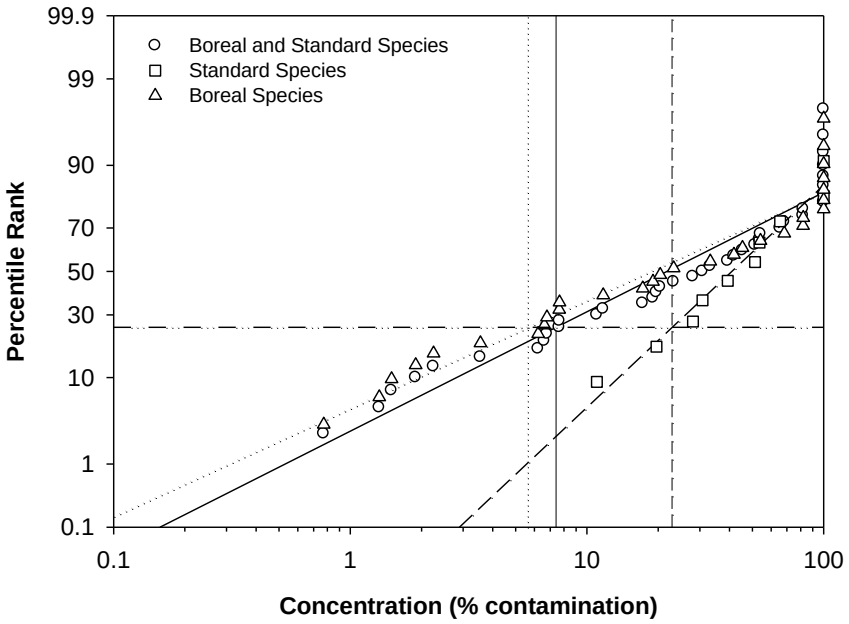

Fig. 5.5. Estimated species sensitivity distributions for boreal (triangles) test species, standard (squares) test species, and all species combined (circles) upon exposure to hydrocarbon-impacted soil, expressed as percent contamination. The line across the y-axis represents the 25th percentile, with corresponding contaminant concentrations identified along the x-axis: 5.6% contamination for the boreal species (dotted line), 23% contamination for the standard species (dashed line) and 7.4% contamination for all species combined (solid line).

5.5.2.2. Salt-impacted soils

With regards to plant growth, all species and endpoints were affected by the salt-impacted soil. Of the standard plant species, *E. lanceolatus* was in general more sensitive than the *T. pratense*; however, the variation observed in the *E. lanceolatus* test precluded any significant difference in response. Of the boreal forest species, *P. banksiana*, *P. glauca*, and *P. mariana* tended to be more sensitive relative to *T. pratense* (Fig. 5.6).

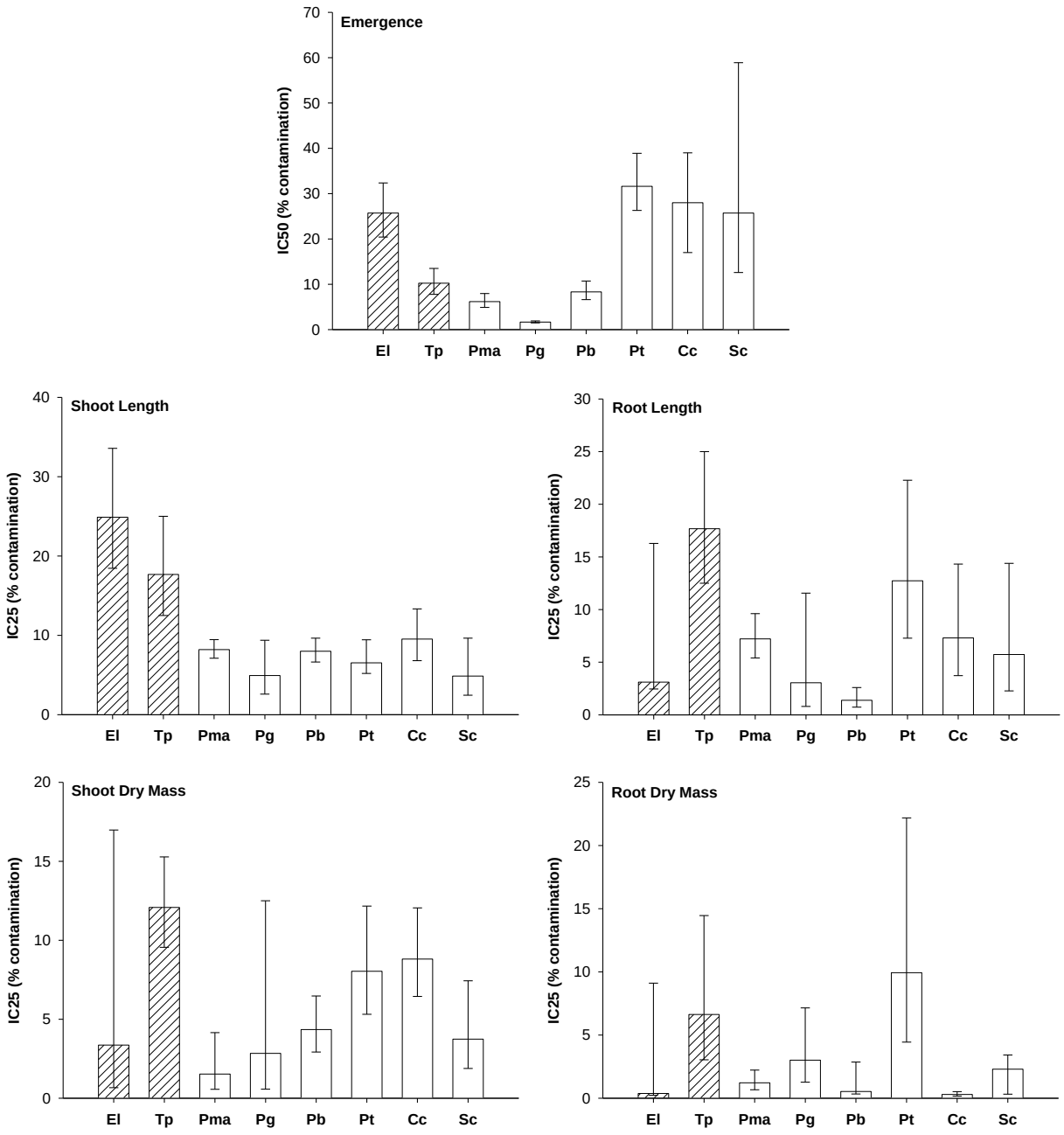

Fig. 5.6. The effect of salt contamination on plant growth, measured by emergence, shoot and root length, and dry biomass. The y-axis represents the EC50 (emergence) or IC25 (plant growth), indicative of the concentration resulting in a 50 or 25% reduction in response relative to the control. Hatched bars are standard test species, and blank bars are the boreal forest test species. Error bars denote the upper and lower 95% confidence limits. Abbreviations are: El = *Elymus lanceolatus* (*n* = 3-6), Tp = *Trifolium pratense* (*n* = 3-6), Pm = *Picea mariana* (*n* = 5-6), Pg = *Picea glauca* (*n* = 5-6), Pb = *Pinus banksiana* (*n* = 5-6), Pt = *Populus tremuloides* (*n* = 5-8), Cc = *Calamagrostis canadensis* (*n* = 5-8)and Sc = *Solidago canadensis* (*n* = 5-9).

Of the earthworm species, the boreal species, *Dd. rubidus* adult survival was significantly inhibited relative to *E. andrei*; juvenile reproduction was also significantly less for *Dd. rubidus* when comparing between IC50s, but was equally affected as *E. andrei* when evaluating IC25s (Fig. 5.7). The derivation of an IC25 was not possible when assessing individual mean juvenile dry mass for the *Dd. rubidus*, as the mean mass increased with increasing test concentration at which juveniles were produced (i.e., 0, 3.1 and 6.3% contamination). When evaluated on a per worm basis, it was observed that the individual mean mass decreased as the number of juveniles increased, possibly creating a density-dependent effect on mass as a test endpoint. However, when evaluating for similar effects with *E. andrei*, no such relationship was observed, and there was no effect (increase or decrease) ($p = 0.83$) in mass between any test concentration (i.e., 0, 3.1, 6.3 and 12.5% contamination) in which juveniles were produced.

The standard and boreal collembolan species were equally affected by the contaminated soil, regardless of test endpoint (Fig. 5.7); and *O. nitens*, was equally affected as the Collembola (Fig. 5.7).

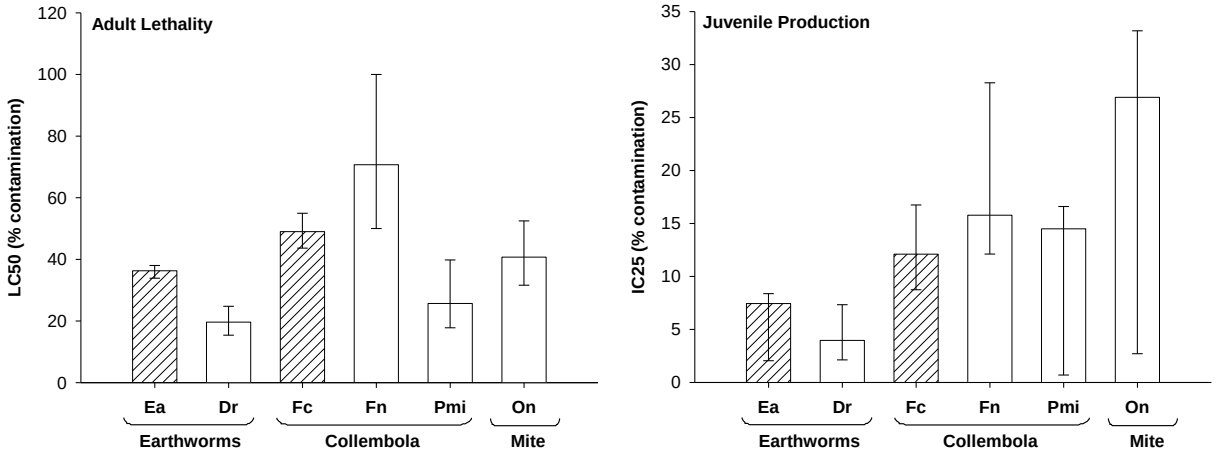

Fig. 5.7. The effect of salt contamination on soil invertebrate reproduction. The y-axis represents the LC50 (lethality) or IC25 (reproduction), indicative of the concentration resulting in a 50 or 25% reduction in response relative to the control. Hatched bars are standard test species, and blank bars are the boreal forest test species. Error bars denote the upper and lower 95% confidence limits. Abbreviations are: Ea = *Eisenia andrei* ($n = 10$), Dr = *Dendrodrilus rubidus* ($n = 3$), Fc = *Folsomia candida* ($n = 3$), Fn = *Folsomia nivalis* ($n = 3$), Pmi = *Proisotoma minuta* ($n = 3$), and On = *Oppia nitens* ($n = 3$).

Similar to the hydrocarbon-impacted soils, ESSDs were generated for each test battery, and electrical conductivity measurements were used to represent the degree of salinity equivalent to the 25th percentile of the distributions. The suite of boreal species was equivalent in sensitivity to the suite of standard test species (Fig. 5.8). For example, the 25th percentile for the boreal species was 2.1% contamination, which equates to 0.22 dS m^{-1} for both the Ah and Ck horizons; the species and endpoints most at risk included only plants: *C. canadensis* root dry mass, *P. banksiana* root length and dry mass, and *P. mariana* root and shoot dry mass. For the standard test species, the 25th percentile was 2.3%, equating to 0.24 and 0.25 dS m^{-1} for the Ah and Ck horizons, respectively; the species and endpoints most at risk included only *E. lanceolatus* root length and dry mass. When combined, the resultant 25th percentile was also 2.3% with *C. canadensis* root dry mass, *P. banksiana* root length and dry mass, *P. mariana* root and shoot dry mass, and *E. lanceolatus* root dry mass falling below this percentile.

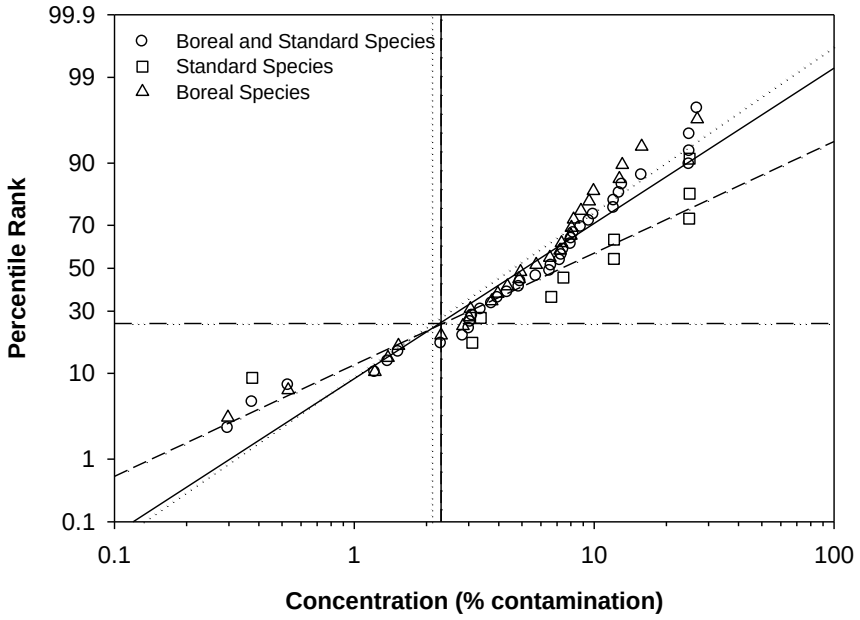

Fig. 5.8. Estimated species sensitivity distributions for boreal (triangles) test species, standard (squares) test species, and all species combined (circles) upon exposure to salt-impacted soil, expressed as a percent contamination of the contaminated soil with reference soil. The line across the y-axis represents the 25th percentile, with corresponding contaminant concentrations identified along the x-axis: 2.1% contamination for the boreal species (dotted line), 2.3% contamination for the standard species (dashed line) and 2.3% contamination for all species combined (solid line).

5.5.2.3. Relayering of soil horizons

The relayering of horizons within test vessels did not impact plant growth, regardless of soil type. However, layering of horizons was found to affect the distribution of soil invertebrates within the test vessel. For *Dd. rubidus* exposed to the salt-impacted soil, adults and juveniles (where reproduction was evident) were generally found within the lower horizons (Fig. 5.9). The reasons for this difference in distribution between horizons are uncertain, but when evaluating for differences in salinity (via electrical conductivity), there was minimal difference between the upper and lower horizons throughout the duration of the test (Fig. 5.10). For the hydrocarbon impacted soils, *Dd. rubidus* adults were also generally found within the lower horizon, however, this may have been a result of lowered PHC levels relative to the upper horizon (Table 5.1); despite chemical variability (only one replicate was analyzed), in most instances, there was little difference in PHC content between the test start and end (Fig. 5.11). *O. nitens* adults and juveniles were consistently found in the upper horizons in both test soils, irrespective of the degree of contamination (Fig. 5.12).

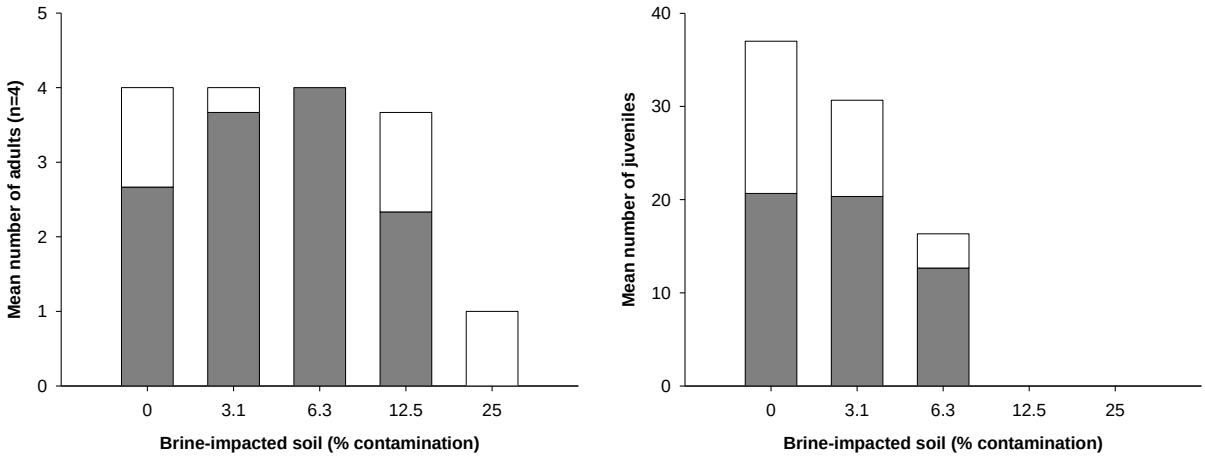

Fig. 5.9. Distribution of adult and juvenile *Dendrodrilus rubidus* between upper (blank bars) and lower (grey bars) soil horizons within the salt-impacted soils (n = 3).

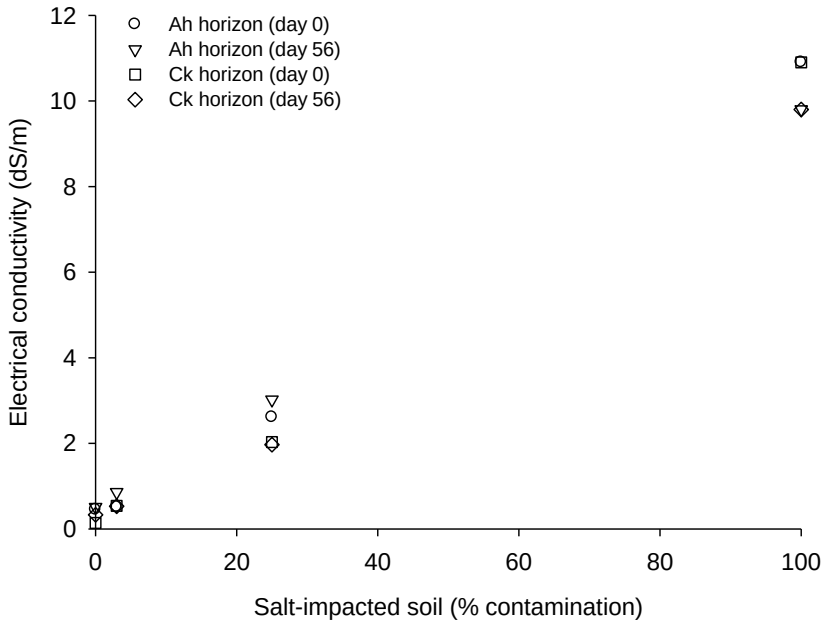

Fig. 5.10. Electrical conductivity measurements (n = 1) of upper (Ah) and lower (Ck) horizons of the salt-impacted soil on days 0 and 56 from an earthworm (*Dd. rubidus*) reproduction test.

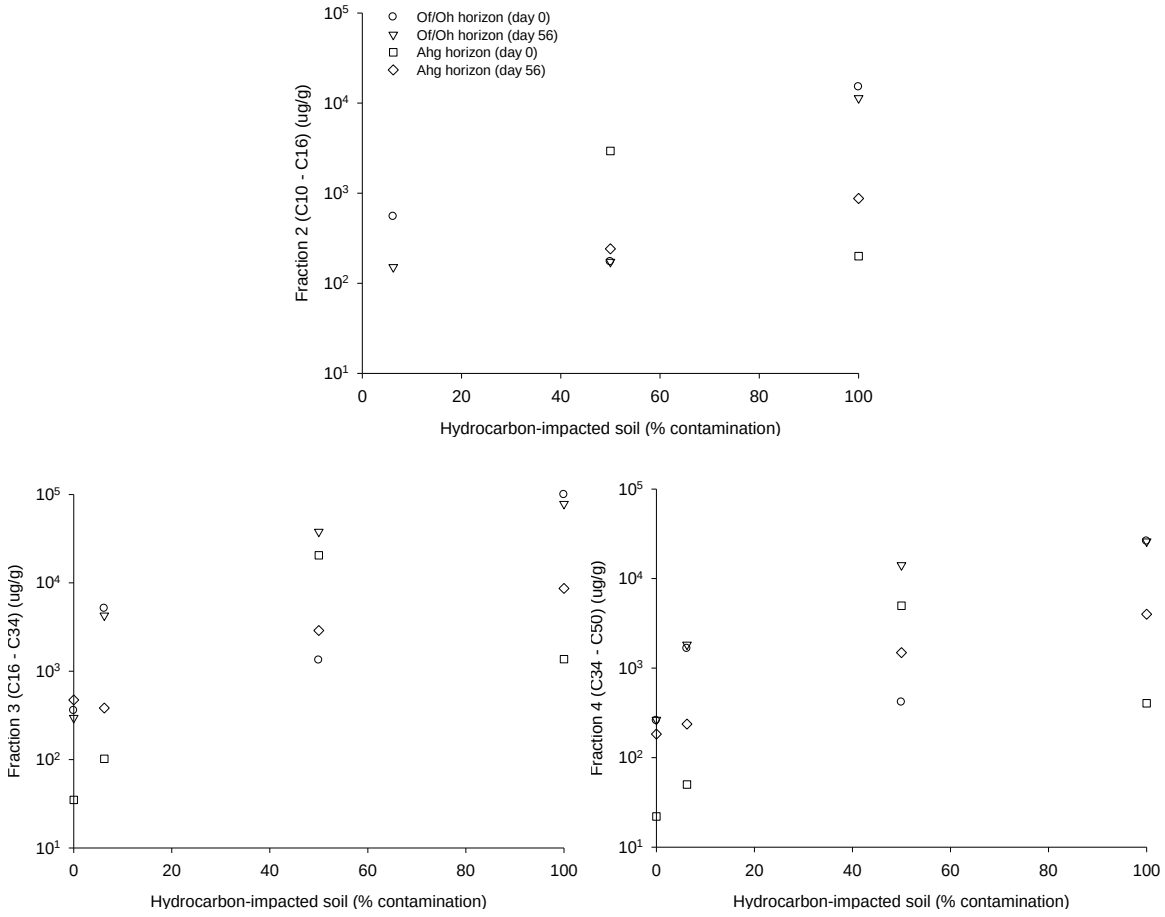

Fig. 5.11. Petroleum hydrocarbon content measured based on equivalent carbon ranges: F Fraction 1 (F1) (C6 to C10), Fraction 2 (F2) (>C10 to C16), Fraction 3 (F3) (>C16 to C34) and Fraction 4 (F4) (>>C34) [1]. Samples (n = 1) were analyzed from the upper (Of/Oh) and lower (Ahg) horizons of the hydrocarbon-impacted soil on days 0 and 56 from an earthworm (*Dd. rubidus*) reproduction test.

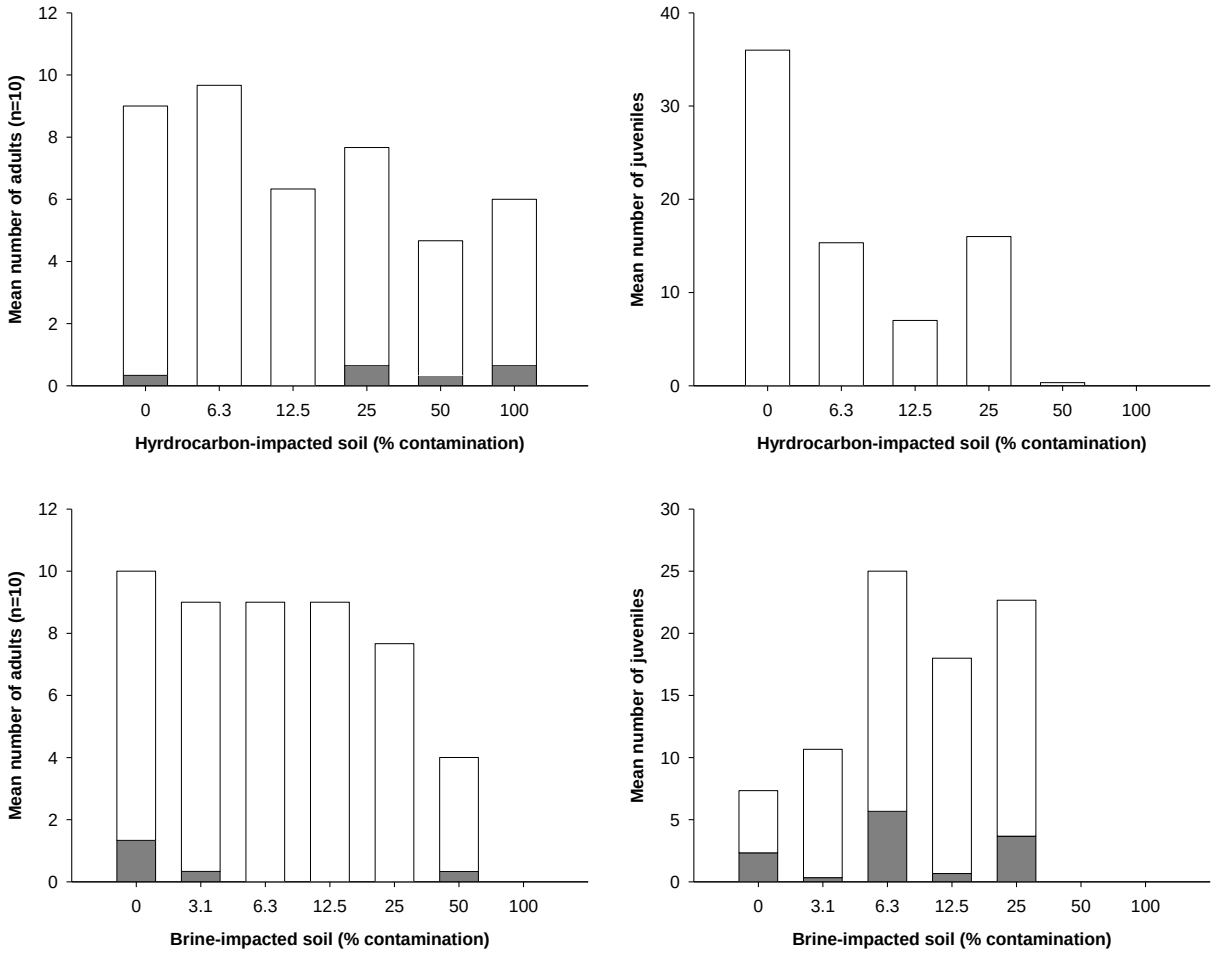

Fig. 5.12. Distribution of adult and juvenile *Oppia nitens* between upper (blank bars) and lower (grey bars) soil horizons within the hydrocarbon and salt-impacted soils (n = 3).

5.6. Discussion

The use of test species relevant to a contaminated site or region under study strengthens the applicability of the resultant toxicity data, particularly for the development of site-specific ecological risk assessments and remedial objectives. The applicability of the boreal species was demonstrated by their comparable performance and test variation relative to the standard test species and methods. In general, the boreal plant test species exhibited acceptable growth such that test variation was <30% for growth endpoints, with few exceptions. Additionally, the majority of test conditions used for the standard plant test species (EC, 2005b) were applicable to the boreal species, with the added necessity of seed stratification to accommodate for their

germination requirements. In order to accommodate for their slower growth rate, the test durations were extended by one to three weeks to attain sufficient plant matter for the measurement endpoints. Although higher than their natural ecological range, the growth temperature of the boreal plants was elevated to encourage maximum plant growth within a short time to balance the practicality of the test. Regional soil temperatures from where the site soils were collected range from 8.3 to 21°C (morning to afternoon) and from 8.5 to 19°C at 5 and 10 cm depth, respectively, throughout the growing season (e.g., May to September) (http://www.climate.weatheroffice.gc.ca/climate_normals/index_e.html).

Similarly, many aspects of the standard invertebrate test methods were applicable to the boreal species, with the exception of the number of organisms used per test vessel, which were optimized for test endpoint measurement (e.g., sufficient reproduction). For comparability, test temperatures were elevated to 20°C for the boreal species, and cultures were reared for approximately eight to twelve months at this temperature post-collection and prior to toxicity testing to prevent potential confounding factors due to temperature stress. Overall test performance was comparable, in that the boreal collembolan and earthworm species were able to meet the same test validity criteria specified for the standard test methods (EC, 2004; EC, 2007); no criteria have been established for oribatid mites. Poor adult survival was observed for *F. nivalis* despite its high reproductive output (i.e., mean of >100 individuals). *F. nivalis* is known to have a widespread nearctic distribution (Christiansen and Bellinger, 1998) and has been recommended for use in forest-related toxicological studies because of its prevalence (Parsons and Parkinson, 1986) and high reproduction potential (Addison, 1996). However, only one study documents the use of this species as a toxicity test species (Addison, 1996); reproductive output was >100 juveniles after 4 weeks of incubation in a control forest soil. In comparison, the reproductive output in this study was 92 ± 49 and 217 ± 46 juveniles in the hydrocarbon and salt reference soils, respectively. *P. minuta* also has wide distribution within Canada (Dodd and Addison, 2010) and has been used more frequently than *F. nivalis* in soil toxicity testing (Dodd and Addison, 2010; Greensdale and Vaughan, 2003; Nursita et al., 2005), although little information is provided on the variation in performance in control soils, nor is the sex of the test species specified. *P. minuta* is a sexually-reproducing species (Massoud and Betsch-Pinot, 1974) (also confirmed in this study) and as such, variation in the ratio of males to females is

likely to yield variation in the reproductive output within a study. As a result, it is recommended that *P. minuta* adults used for reproduction studies utilize age-synchronized individuals, with a 1:1 ratio of males to females (for a total of 10 adults per test vessel).

For the oribatid mite, *Oppia nitens*, adult survival was comparable to the Collembola (i.e., >70%) in all reference soils, although juvenile production was low (i.e., <100 individuals). This is to be expected and is reflective of the life-history in that oribatid mites generally have slower metabolism, lower reproduction, and a longer life span with a lowered capacity for population increase (Norton and Behan-Pelletier, 2009); however, these characteristics are counter-balanced by their increased abundance and prevalence within soil systems. Test variation associated with juvenile production can be reduced by age-synchronizing the adult test population (Chapter 3), as well as increasing the number of adults used at the test start (e.g., to 20 individuals).

This study also demonstrated the utility of using boreal test species to assess contaminated boreal soils, and in the case of the hydrocarbon-impacted soils, the boreal species were more affected by the contamination relative to the standard species. Plant toxicity varied with species and endpoint; however, *C. canadensis* and *P. tremuloides* were generally more sensitive to the hydrocarbon-impacted soils, all plants considered. Although highly acidic (< pH 4.0), the reduction in growth was likely not attributed to soil pH as these two species are tolerant of acidic (e.g., > pH 3.0) to neutral and slightly alkaline (e.g., ~ pH 8.0) soil conditions (Howat, 2000). Even though test durations were extended for the boreal species to accommodate for their growth rate, differences in toxicity due to test duration was likely not a major contributing factor, as in some cases, test endpoints for the boreal plants (e.g., *P. banksiana* 35-d test) were less sensitive than the standard test species. The toxicity of PHCs in soil to plants varies with species and endpoint (Salanitro et al., 1997), and because it is also dependent upon soil type and petroleum source, a comparison to other studies is difficult. However, consistent with other studies (Dorn et al., 1998; Cermak et al., 2010), greater sensitivity was demonstrated by soil invertebrate sublethal endpoints, relative to plant endpoints. Of the soil invertebrates, collembolan and oribatid mite reproduction were equally affected. This is likely a result of similar exposure pathways (i.e., dermal and oral contact with soil) as well as the mechanism of

action for hydrocarbon toxicity, which for many soil invertebrates, consists of non-polar narcosis or disruption of cellular membranes (Jager et al., 2000; Sverdrup et al., 2001). A comparison between sublethal effects for the earthworm species, *Dd. rubidus* and *E. andrei*, was not possible as although adults survived, reproduction was not evident in either the reference or contaminated soils. It is possible this response was due to other factors rather than contaminant sensitivity, especially given the lack of response in the control soils. Although *Dd. rubidus* exhibits tolerance to low pH soils (Edwards and Bohlen, 1996), a decrease in cocoon production and hatching success has been demonstrated under laboratory conditions in soil < pH 4.5 (Rundgren and Nilsson, 1997). Additionally, both earthworm cultures were reared in a pH-neutral soil substrate prior to use in soil testing; therefore, it is possible that the abrupt change in soil pH between the culture substrate and test soil may have also contributed to the lack of reproduction.

Salt toxicity to plants was species and endpoint-specific, with no discernible species being consistently more sensitive than another. Boreal plant tolerance to salinity (based on electrical conductivity (EC) as a measurement of saline conditions) is similar among the species, with a tolerance ranging from ~1.0 to 11 dS m^{-1}, with the exception of *S. canadensis*, which exhibits a slightly higher tolerance from ~15 to 31 dS m^{-1} (Howat, 2000). Of the standard test species, *E. lanceolatus* demonstrates some tolerance to moderate salinity, with an EC ranging from 2.0 to >9.0 dS m^{-1} (Howat, 2000). All plant species considered, 25% inhibition in plant growth (i.e., measured as shoot and root length and dry biomass) occurred between EC estimates of 0.03 dS/m for *C. calamagrostis* root dry mass to 2.7 dS m^{-1} for *T. pratense* root length.

Salt toxicity to invertebrates was also similar between standard and boreal test species. Of the boreal invertebrate species, only *Dd. rubidus* survival was significantly less than *E. andrei* with effects on reproduction equivalent between the two species. The collembolan species were also equally affected, with the oribatid mite equally affected as the Collembola. The similarity in response between the soil invertebrates may be a reflection of the mode of action of excessive soil salinity in that it can disrupt osmoregulation (i.e., loss of body water, resulting in greater ionic concentration of salts), and consequently, survival, growth and reproduction (Witteveen et al., 1987; Fischer and Molnár, 1997). Prior research using an agricultural saline soil demonstrated a significant decrease in *F. candida* juvenile production at an EC >1.0 dS m^{-1}, and

for the earthworm species, *Eisenia fetida*, adult survival was significantly affected at >0.92 dS m^{-1} with no cocoon production occurring at >0.52 dS m^{-1} (Owojori et al., 2009). Similarly, the results of this study demonstrated a 25% reduction in the production of juveniles at these respective EC, dS m^{-1}, values: 0.43 (*Dd. rubidus*), 0.80 *(E. andrei*), 1.3 (*F. candida*), 1.4 (*P. minuta*), 1.7 (*F. nivalis*), and 2.9 (*O. nitens*).

The experimental design implemented in this study incorporated the collection and relayering of individual soil horizons within the test vessel. This was done to more closely represent true conditions within the environment, rather than mixing two distinctly different soil horizons that might occur if collecting to depth, with no regard to horizon differences. The use of the layered approach was acceptable for plants, as plant growth was unaffected by this design, and root growth and development is expected in some cases to span more than one horizon in depth. However, the design was inappropriate for the assessment of effects on invertebrate species, as species tended to reside in one horizon over another. For example, a preference (or avoidance) of upper or lower horizons was observed for *Dd. rubidus* although the mechanism for this behaviour is uncertain and requires further study. In the case of the *O. nitens*, adults and juveniles were consistently located in the upper organic horizons, irrespective of the degree of contamination. Oribatid mite community structure is predominantly influenced by humus form (Maraun and Scheu, 2000), and in natural soils, will be also be influenced by the type and quality of organic matter and fungal biomass, which serves as a primary food source (Chapter 3). With regards to the collembolan species, a layered design was difficult, given the rapid mobility and redistribution of these organisms between horizons upon disturbance to the test vessel at the time of test processing. As a result, although multiple horizons might be collected, individual soil horizons should be assessed separately (rather than layered) when using soil invertebrates as a test species. This would minimize the confounding effect of horizon preference (or avoidance) that might lead to an over or underestimation of the toxic potential of contaminated soils.

6. SYNTHESIS AND CONCLUSIONS

The research presented in this thesis contributes to the development of soil toxicity test methods applicable to the assessment of contaminated soils in boreal regions, using a battery of species and soils prevalent and applicable to these regions. Boreal regions account for over half of Canada's landmass, and resources within these regions significantly contribute to Canada's economy through forestry, mining, and oil and gas activities. The inadvertent release of pollutants from industrial activities within these regions stress the need for realistic ecological risk assessments, as well as the derivation of realistic remediation objectives, which can be attained through the use of standardized soil toxicity test methods. Despite the geophysical and economic significance of boreal regions, standardized soil toxicity test methods are lacking in general that specifically address these northern non-temperate ecosystems.

Boreal forest soils are uniquely characterized by their well-developed horizon structure, with thick forest floor and organic layers covering mineral soils (Fisher and Binkley, 2000); as such, the conditions and soil types within these regions give rise to plant and soil invertebrate species that differ from those currently represented by standard test methods, which are typically representative of temperate regions. The selection and use of species requires a balance between ecological relevancy and representation of the structural and functional diversity present within a healthy soil community, but also practical considerations for the functionality of the test (van Gestel, 1998; Stephenson, 2003). Such factors were taken into consideration for the selection of the test battery presented within this thesis, with particular emphasis on the use of oribatid mites. Oribatid mites are the most abundant and diverse microarthropod present in boreal forest soils (Crossley and Bohnsack, 1960; Walter, 1985; Heneghan et al., 1999), significantly contributing to soil formation processes (Singh et al., 1996; Johnston and Crossley, 2002; Coleman et al., 2004). Despite this information, and the demonstrative fact that they are good indicators of soil quality (e.g., Andrés, 1999; Behan-Pelletier, 1999), the number of ecotoxicity tests that

incorporate oribatid mites in the assessment of contaminated soils are few relative to that of other soil invertebrates (e.g., lumbricids, enchytraeids, Collembola, isopods, gastropods).

6.1. *Oppia nitens* (C.L. Koch) as a Suitable Soil Toxicity Test Species

Overall, the research presented in this thesis demonstrated the applicability and identified some limitations of using the oribatid mite, *Oppia nitens*, as a potential test species in soil ecotoxicity testing. The organism can be easily cultured under laboratory conditions for a prolonged period of time without supplementation with field-collected specimens. Because of the unique morphological characteristics exhibited by oribatid mites between life stages, test populations can be age-synchronized with the harvest of tritonymph instars that are allowed to mature to adults. The performance of *O. nitens* across multiple reference soils also suggests that *O. nitens* can tolerate a relatively large soil pH range (e.g., 3.9 to 7.5 for the field-collected soils), as when evaluating the influence of the soil characteristics on reproduction, soil pH was not a significant influential factor. In general, adult survival was not influenced by the soil characteristics studied across reference soils (i.e., ammonia (NH_3), nitrite (NO_2), pH, phosphorous (P), organic matter (OM) content, carbon:nitrogen (C:N) ratio, sand, silt, clay and sodium adsorption ratio (SAR)); however, reproduction was limited by soil organic matter content. This may limit the applicability of a reproduction test to certain soil types (e.g., sub-surface or mineral soils) unless these sub-optimal soils are amended to increase the soil organic matter content. Research demonstrates that oribatid mite community structure is predominantly driven by humus form, rather than other parameters such as soil acidity, humidity, and temperature (Maraun and Scheu, 2000). In addition, organic matter content and quality varies and is limiting across horizons rather than within horizons (Klironomos and Kendrick, 1995). As organic matter content was found to affect reproduction, the individual soil horizons within a site should be taken into consideration when interpreting test results. This is especially important when evaluating the performance of the test species between contaminated and non-contaminated (i.e., reference) soils. The addition of peat as an organic substrate in soil toxicity testing using a fresh chemical amendment also altered the toxicity of the substance to adult survival, but not for reproduction. Therefore, further studies are required to investigate the effect

of organic matter content and type on the performance of *O. nitens*, particularly in aged contaminated soils.

6.2. The Toxicokinetics of Phenanthrene on *Oppia nitens*

Several studies demonstrate the sensitivity of oribatid mites to hydrocarbon pollution in soil (Parmelee et al., 1993; Lebrun and van Straalen, 1995; Erstfeld and Snow-Ashbrook, 1999; Blakely et al., 2002), some of which may include various types of polycyclic aromatic hydrocarbons (PAHs). Polycyclic aromatic hydrocarbons are also likely to partition within the organic components of soil, particularly horizons rich in organic matter (Jager et al., 2000; Krauss et al., 2000; Zhang et al., 2006). Because oribatid mites are known to primarily inhabit organic-rich horizons and soils, the effect and bioaccumulation potential of a model substance (phenanthrene) to *O. nitens* in soil was assessed. The evaluation of phenanthrene in artificial soil yielded adverse effects on adult survival (LC50 = 388 $mg \cdot kg^{-1}$, 95% CL: 330 - 446) and juvenile production (IC25 = 48 $mg \cdot kg^{-1}$, 95% CL: 15 - 146; IC50 = 95 $mg \cdot kg^{-1}$, 95% CL: 26 - 345)at levels similar to those observed by other soil invertebrates (e.g., Collembola, earthworms and enchytraeids). Exposure of *O. nitens* to phenanthrene resulted in consistent accumulation across time for the test concentrations (i.e., 100, 200 and 400 mg kg^{-1}); however, steady-state was not reached during the four-week exposure period. The uptake phase was biphasic, characterized by a gradual, followed by rapid uptake. The initial gradual uptake was likely a result of cuticular (dermal) sorption processes, followed by other contributing processes (e.g., dietary uptake). Once transferred to clean soil, the organisms were able to eliminate the chemical, however, the phenanthrene was not completely eliminated in that residual levels (equivalent to levels observed during the gradual initial accumulation) remained by the end of the elimination phase, indicative of dermal sorption processes significantly contributing to the overall accumulation processes. High replicate variability was also observed throughout the uptake and elimination phases, and were likely a result of low sample size, although phenanthrene could none the less be detected at levels above the method detection limit. Bioaccumulation increased with increasing exposure concentration, but the resultant bioaccumulation factors (BAFs) were relatively low (BAF_k < 0.031), indicative of limited trophic transfer and biomagnification for this species. Further tests, using PAHs are warranted in order to determine minimum sample size for analysis, but also to

confirm whether the observed trends hold true, given that very few studies have been conducted using oribatid mites despite their demonstrated sensitivity to hydrocarbon pollution in general.

6.3. Evaluation of a New Battery of Toxicity Tests for Boreal Forest Soils

The primary focus of the research presented herein encompassed the development of a new oribatid mite toxicity test, which was successfully applied to boreal forest soils. However, a comparison of the sensitivity of *O. nitens* to that of other boreal species was warranted. As a result, toxicity studies, using field-collected reference and contaminated (hydrocarbon- and salt-impacted) soils, were completed using an expanded suite of boreal species encompassing springtails (Collembola), earthworms and boreal tree and understory plants. The sensitivity of the boreal species was then compared to that of currently published standard soil toxicity test species (earthworms, Collembola and agronomic plants) to determine whether the boreal species were more or less sensitive to the contamination.

The boreal test species and toxicity test methods used in the present study demonstrated the applicability of using ecologically-relevant test species, which were in some instances, more sensitive to soil contamination, in comparison to standard test species. The results of the research presented a test battery representative of different trophic levels using species naturally occurring within Canadian boreal regions, and in some cases, used for reclamation purposes (e.g., *P. glauca*, *P. tremuloides*, and *P. banksiana*) (Khasa et al., 2005). For the oribatid mite, *O. nitens*, adult survival was comparable to the Collembola (i.e., >70%) in the reference soils; however, juvenile production was relatively low (i.e., <100 individuals). This is to be expected and is reflective of the life-history in that oribatid mites generally have slower metabolism, lower reproduction, and a longer life span with a lowered capacity for population increase (Norton and Behan-Pelletier, 2009); however, these characteristics are counter-balanced by their increased abundance and prevalence within soil systems. Similar to the initial studies conducted with *O. nitens*, test variation associated with juvenile production was high. This can be reduced by age-synchronizing the adult test population, as well as increasing the number of adults used at the test start (e.g., to 20 individuals).

Although the toxicity of petroleum hydrocarbons is dependent upon soil type and petroleum source, greater sensitivity was demonstrated by soil invertebrate sublethal endpoints, relative to plant endpoints, which is consistent with other studies (Dorn et al., 1998; Cermak et al., 2010). Of the soil invertebrates, collembolan and oribatid mite reproduction were equally affected, likely a result of similar exposure pathways (i.e., dermal and oral contact with soil), as well as the narcotic mode of action for hydrocarbon toxicity (Jager et al., 2000; Sverdrup et al., 2001). Estimated species sensitivity distributions (ESSDs) were also generated to determine whether the boreal and standard test battery of species exhibited differences in their overall sensitivity to the contaminated soil. The resultant distribution of species sensitivity demonstrated greater sensitivity of the boreal species to the petroleum hydrocarbon-impacted soil, relative to the suite of standard test species.

Salt toxicity to plants was species and endpoint-specific, with no discernible species being consistently more sensitive than another. The effect of soil salinity on plant growth seems to vary widely among species (Table 6.1), with the standard test species, *E. lanceolatus* demonstrating some tolerance to moderate salinity (2.0 to >9.0 dS m^{-1}) (Howat, 2000). Of all the plant species considered within this research, a 25% inhibition in plant growth (i.e., measured as shoot and root length and dry biomass) occurred between electrical conductivity estimates of 0.03 dS/m for *C. calamagrostis* root dry mass to 2.7 dS m^{-1} for *T. pratense* root length (Table 6.1). In general, plant growth becomes affected when electrical conductivity exceeds 4 dS m^{-1} (Howat, 2000; Lilles et al., 2012).

Salt toxicity to the invertebrates was also similar between the standard and boreal test species. The similarity in response between the soil invertebrates may be a reflection of the mode of action of excessive soil salinity in that it can disrupt osmoregulation (i.e., loss of body water, resulting in greater ionic concentration of salts), and consequently, survival, growth and reproduction (Witteveen et al., 1987; Fischer and Molnár, 1997). Although relatively little data is available, the salt tolerances observed by these species in soil were consistent with other reported sensitivities (Table 6.2).

In contrast to the petroleum hydrocarbon-impacted soils, the ESSDs demonstrated that both the boreal and standard suite of test species were equally sensitivity to the salt-impacted soils.

Table 6.1. Summary of soil salinity tolerance ranges for current standardized test species (EC, 2005) and recommended boreal test species.

Species (common name)	Electrical Conductivity Tolerance (dS m^{-1})	SAR† Tolerance Range	Reference
Currently-used Standard Test Species (EC, 2005)			
Elymus lanceolatus (northern wheatgrass)	2 to > 9		Howat (2000)
	0.041 to 2.7	0.24 to 16	Chapter 5‡
Festuca rubra (creeping red fescue)	20 to 24		Howat (2000)
Trifolium pratense (red clover)	0.72 to 2.7	4.2 to 16	Chapter 5‡
Recommended Boreal Test Species			
Calamagrostis canadensis (bluejoint reedgrass)	15 to 31		Howat (2000)
	0.032 to 1.0	0.19 to 6.1	Chapter 5‡
Solidago canadensis (Canada goldenrod)	0.25 to 0.62	1.5 to 16	Chapter 5‡
Betula papyrifera (paper birch)	2.4 to < 6.4		Howat (2000)
Picea glauca (white spruce)	8.8 to 15	0 to 26	Howat (2000)
	0.31 to 0.53	1.8 to 3.2	Chapter 5‡
Picea mariana (black spruce)	0.21 to 7.1	< 0.20	Howat (2000)
	0.13 to 0.89	0.78 to 5.3	Chapter 5‡
Pinus banksiana (jack pine)	1.0 to 6.3	0 to 7.2	Howat (2000)
	0.057 to 0.86	0.34 to 5.1	Chapter 5‡
Populus tremuloides (trembling aspen)	0.98 to 7.9	0 to 26	Howat (2000)
	0.71 to 1.4	4.2 to 8.2	Chapter 5‡

†SAR sodium adsorption ratio

‡Results presented in this table from Chapter 5 have been determined based on the SAR measured in reference and contaminated salt-impacted soils, and calculated from the 25% inhibitory effect levels (based on the percent contamination derived from the measured SAR).

Table 6.2. Summary of soil salinity tolerance ranges for current standardized test species (EC, 2004; EC, 2007) and recommended boreal test species.

Species and endpoint	Electrical Conductivity Tolerance Range (dS m^{-1})	SAR† Tolerance Range	Reference
Currently-used Standard Test Species (EC, 2003; EC, 2007)			
Folsomia candida			
Juvenile production	<1.3	7.8	Chapter 5‡
Juvenile production	<1.0		Owojori et al. (2009)
Eisenia fetida			
Adult survival	<0.92		Owojori et al. (2009)
Cocoon production	<0.52		Owojori et al. (2009)
Eisenia andrei			
Juvenile production	<0.80	4.8	Chapter 5‡
Recommended Boreal Test Species			
Folsomia nivalis			
Juvenile production	<1.7	10	Chapter 5‡
Proisotoma minuta			
Juvenile production	<1.4	8.4	Chapter 5‡
Oppia nitens			
Juvenile production	<2.9	17	Chapter 5‡
Dendrodrilus rubidus			
Juvenile production	<0.43	2.5	Chapter 5‡

†SAR sodium absorption ratio

‡Results presented in this table from Chapter 5 have been determined based on the SAR measured in reference and contaminated salt-impacted soils, and calculated from the 25% inhibitory effect levels (based on the percent contamination derived from the measured SAR).

The evaluation of boreal species and soils also took into consideration the use of distinct soil horizons, as in some cases, horizon differentiation can remain unperturbed despite the occurrence of a pollution event (e.g., surface spill). This is also in contrast to the traditional collection of soils, for example, from arable regions, where the soil is tilled, and horizons are disturbed resulting in a depth-based collection and loss of horizon structure. To maintain horizon-specific differences, individual soil horizons were collected from impacted sites and relayered within the test vessels. The layering of horizons was feasible from the initial collection to reassembly for testing in the laboratory, and plant growth was unaffected by this design. However, soil invertebrates demonstrated a preference or avoidance tendency for one horizon over another. The preference or avoidance for a particular horizon (e.g., for a soil type or chemical avoidance) can confound test results and the interpretation of the toxic potential of a site. As a result, for soil invertebrates, individual soil horizons should be assessed separately.

6.4. Concluding Thoughts and Recommendations

The compilation of research presented herein provides the basis for the standardization of ecologically-relevant test species and methods for the assessment of contaminated soils in boreal regions. Specifically, oribatid mites represent a significant niche within soils, but remain under-represented in ecotoxicology. *Oppia nitens,* is not only representative of the ecological niche occupied by oribatid mites, but has proved to be a good candidate species for a number of forest soils. The use of species represents a balance between ecological relevance and practical considerations (Table 6.3).

Table 6.3. Summary of ecological and practical considerations for the use of *Oppia nitens* as a soil test species.

Ecological Relevancy	Practical Considerations
• Native, abundant and relevant to soil exposure • Demonstrates varying environmental stress (relatively insensitive) versus pollutant (sensitive) tolerance • Taxonomy is definite	• Relatively rapid life cycle of 3-4 weeks • Sexually reproducing • Easily cultured with physiologically distinct life stages (good for age-synchronization) • Test conditions similar to existing standard soil test methods for soil invertebrates, with little soil required

Although adult survival as a test endpoint was highly insensitive to soil characteristics (that might represent environmental stress factors, such as soil pH, moisture content, etc.), reproduction was limited by reduced soil organic matter content. Further studies are warranted that explore the degree to which juvenile production is affected not only by organic matter content, but also by organic matter type. Another limiting factor worthy of further exploration is the high degree of test variation observed with the reproductive test endpoint (i.e., number of juveniles produced). However, this could be mitigated by conducting further research on the narrowing the age-synchronization (e.g., selection of newly eclosed adults that are amber in colour) within 1-2 days, and allowing maturation for a pre-defined duration before addition to the test soil. Reproduction could also be enhanced by optimizing the number of adults per test vessel, for example, these studies used 10 adults per test vessel for the soil toxicity tests, but this should be increased to 20 adults per test vessel. An increase in the adult population might

increase the number of juvenile produced such that replicate variability could be reduced. Similarly, the evaluation of test duration might also affect the level of reproduction. All tests conducted so far have used a 28-d test duration, based on the 3-4 weeks required for egg laying and maturation to at least the tritonymph life stage. An extension of the test by one additional week may increase juvenile production, but may also allow for maturation of immatures to adult form such that distinguishing between the adult and progeny population might prove difficult. It is also quite difficult to pre-determine the gender of *O. nitens* adults added to the test vessel, as this species does not demonstrate sexual dimorphism; therefore, it would be quite interesting to determine the gender ratio relative to the number of juveniles produced. The gender of the test species cannot be determined alive, and therefore, gender identification could be conducted at the end of the test when the organisms are heat-extracted from the test vessel. The use of heat extraction to collect surviving adult and immature stages should also be validated, particularly in the assessment of immature stages. It is likely that if immature forms are under-going maturation and ecdysis (moulting), they will not be able to move through the soil as a result of the applied heat and desiccation of the soil, into the extraction vessels for subsequent enumeration. Further studies should also incorporate the use of power analysis, as more data become available, as this would allow for the optimization of replicate requirements to reduce or minimize test variation.

However, since the introduction of this species as a representative oribatid mite species for soil toxicity tests (Chapter 2: Princz et al., 2010), the number of studies evaluating the effects of chemicals on *O. nitens* has increased, including the use of an avoidance behaviour as a screening tool (Owojori et al., 2011; Owojori et al., 2012), to the recommendation of this and other species described in Chapter 5 (Princz et al., 2012) for the assessment of acidic metal-contaminated soils (Chapman et al., 2013). Indeed, the under-representation of soil arthropods is recognized, despite their abundance within the field, stressing the need for further development and standardization efforts (van Gestel, 2012). The data and recommendations provided herein this thesis will provide the foundation for such efforts relative to the use of *O. nitens* as a representative oribatid mite species in the assessment of contaminants in soils.

The suite of test organisms proposed within this thesis has striven to capture structural and functional complexity of boreal soil systems, using species and techniques applicable to a laboratory setting. The proposed species (plants, earthworms, Collembola and oribatid mites) represent different trophic levels, allowing for the consideration of various routes of uptake as well as varying toxicokinetic processes such as biotransformation or detoxification thus accounting for species- and endpoint-sensitivity. The ability to use a relevant test battery supports the development of realistic site-specific ecological risk assessments and remediation programs applicable to affected sites, particularly because the test suite encompasses species native to affected boreal regions, and species that might be used as part of reclamation strategies.

7. REFERENCES

Achazi RK, Flenner C, Livingstone DR, Peters LD, Schaub K, Scheiwe E. 1998. Cytochrome P450 and dependent activities in unexposed and PAH-exposed terrestrial annelids. *Comp Biochem Physiol C* 121:339-350.

Achuba FI. 2006. The effect of sublethal concentrations of crude oil on the growth and metabolism of Cowpea (*Vigna unguiculata*) seedlings. *Environmentalist* 26:17-20.

Adamski Z, Bloszyk J, Bruin J, Ziemnicki K. 2007. Non-omnia moriantur - toxicity of mancozeb on dead wood microarthropod fauna. *Exp Appl Acarol* 42:47-53.

Addison JA. 1996. Safety testing of tebufenozide, a new molt-inducing insecticide, for effects on nontarget forest soil invertebrates. *Ecotox Environ Safe* 33:55-61.

Addison JA. 2009. Distribution and impacts of invasive earthworms in Canadian forest ecosystems. *Biol Invasions* 11:59-79.

Addison JA, Holmes SB. 1995. Comparison of forest soil microcosm and acute toxicity studies for determining effects of fenitrothion on earthworms. *Ecotox Environ Safe* 30:127-133.

Addison JA, Otvos IS, Battigelli JP, Conder N. 2006. Does aerial spraying of *Bacillus thuringiensis* subsp. *kurstaki* (Btk) pose a risk to nontarget soil microarthropods? *Can J For Res* 36:1610–1620.

Al-Assiuty AIM, Khalil MA. 1995. The influence of insecticide-pheromone substitution on the abundance and distributional pattern of soil oribatid mites. *Exp Appl Acarol* 19:399–410.

Al-Assiuty AIM, Khalil MA, Abdel-Lateif HM. 2000. Effects of dry sludge application on soil microarthropod communities in a reclaimed desert ecosystem. *Pedobiologia* 44:567-578.

Alberta Environment. 2007. *Tier 2 Ecocontact Guideline Derivation Protocol*. Environmental Policy Branch, Edmonton, Alberta, Canada.

Alexander M. 1995. How toxic are toxic chemicals in soil? *Environ Sci Technol* 29:2713-2717.

Alexander M. 2000. Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environ Sci Technol* 34:4259-4265.

Alkio M, Tabuchi TM, Wang X, Colon-Carmona A. 2005. Stress responses to polycyclic aromatic hydrocarbons in *Arabidopsis* include growth inhibition and hypersensitive response-like symptoms. *J Exp Bot* 56:2983-94.

Al-niemi TS, Campbell WF, Rumbaugh MD. 1992. Response of alfalfa cultivars to salinity during germination and post germination growth. *Crop Sci* 32:979-980.

Amellal N, Portal J-M, Berthelin J. 2001. Effect of soil structure on the bioavailability of polycyclic aromatic hydrocarbons within aggregates of a contaminated soil. *Appl Geochem* 16:1611-1619.

American Society of Testing and Materials (ASTM). 1999. Standard Guide for Conducting Terrestrial Plant Toxicity Tests, E1963-98. In *Annual Book of ASTM Standards - Biological Effects and Environmental Fate; Biotechnology; Pesticides, Vol. 11.05*. American Society for Testing and Materials, Philadelphia, Pennsylvania, USA, pp. 1481-1500.

Amorim MJB, Oliveira E, Teixeira AS, Gravato CS, Loureiro S, Guilhermino LC, van Gestel CAM, Soares AMVM. 2011. Toxicity and bioaccumulation of phenanthrene in *Enchytraeus albidus* (Oligochaeta: Enchytraeidae). *Environ Toxicol Chem* 30:967-972.

Andrés P. 1999. Ecological risks of the use of sewage sludge as fertilizer in soil restoration: effects on the soil microarthropod populations. *Land Degrad Dev* 10:67-77.

Andrés P, Mateos E, Tarrasón D, Cabrera C, Figuerola B. 2011. Effects of digested, composted, and thermally dried sewage sludge on soil microbiota and mesofauna. *Appl Soil Ecol* 48: 236-242.

Angell RA, Kullman S, Shrive E, Stephenson G, Tindal. 2012. Direct soil contact values for ecological receptors exposed to weathered petroleum hydrocarbon (PHC) Fraction 2. *Environ Toxicol Chem* 31:2536-2544.

Anielski MP, Wilson SJ. 2003. *Counting Canada's Natural Capital: Assessing the Real Value of Canada's Boreal Ecosystems*. Canadian Boreal Initiative, Ottawa, Canada, 90 pp.

Arnold RE, Hodson ME, Langdon CJ. 2008. A Cu tolerant population of the earthworm *Dendrodrilus rubidus* (Savigny, 1862) at Coniston Copper Mines, Cumbria, UK. *Environ Pollut* 152:713-722.

Ayers AD, Brown JW, Widleigh CH. 1952. Salt tolerance of barley and wheat in soil plots receiving several salinization regimes. *Agron J* 44:307-310.

Battigelli JP, Spence JR, Langor DW, Berch SM. 2004. Short-term impact of forest soil compaction and organic matter removal on soil mesofauna density and oribatid mite diversity. *Can J For Res* 34:1136-1149.

Beck L, Römbke J, Ruf A, Prinzing A, Woas S. 2004. Effects of diflubenzuron and *Bacillus thuringiensis* var. *kurstaki* toxin on soil invertebrates of a mixed deciduous forest in the Upper RhineValley, Germany. *Eur J Soil Biol* 40:55-62.

Bedard-Haughn A. 2011. Gleysolic soils of Canada: genesis, distribution, and classification. *Can J Soil Sci* 91:763-779.

Begum F, Karmoker JL, Fattach QA, Maniruzzaman AFM. 1992. The effects of salinity on germination and its correlation with K+, Na+, and Cl- accumulation in germinating seeds of *Triticum aestivum* L. var. Akbar. *Plant Cell Physiology* 1009-1014.

Behan VM, Hill SB, McE Kevan DK. 1978. Effects of nitrogen fertilizers, as urea, on Acarina and other arthropods in Quebec black spruce humus. *Pedobiologia* 18:249-263.

Behan-Pelletier VM. 1999. Oribatid mite biodiversity in agroecosystems: role for bioindication. *Agric Ecosyst Environ* 74:411-423.

Behan-Pelletier VM, Eamer B. 2004. Diversity of Oribatida in Canada. *http://www.cbif.gc.ca/spp_pages/mites/phps/index_e.php*

Behan-Pelletier VM, Hill SB. 1983. Feeding habits of sixteen species of Oribatei (Acari) from an acid peat bog, Glenamoy, Ireland. *Rev Ecol Biol Sol* 20:221-267.

Beirkens J, Klein G, Corbisier P, van den Heuvel R, Verschaeve L, Weltens R, Schoeters G. 1998. Comparative sensitivity of 20 bioassays for soil quality. *Chemosphere* 37:2935-2947.

Belfroid AC, Sijm DTHM, van Gestel CAM. 1996. Bioavailability and toxicokinetics of hydrophobic aromatic compounds in benthic and terrestrial organisms. *Environ Rev* 4:276-299.

Bengtsson G, Gunnarsson T, Rundgren S. 1986. Effects of metal pollution on the earthworm *Dendrobaena rubida* (Sav.) in acidified soils. *Water Air Soil Poll* 28:361-383.

Bengtsson G, Rundgren S. 1988. The Gusum case: a brass mill and the distribution of soil Collembola. *Can J Zoo*l 66:1518-1526.

Billeret M, Berny P, Mazallon M, Buronfosse T. 2000. Bioavailability of polycyclic aromatic hydrocarbons and polychlorinated biphenyls in rats from naturally contaminated soils - preliminary evaluation of the influence of soil parameters. *Environ Toxicol Chem* 19:2614-2620.

Bindesbøl A-M, Bayley M, Damgaard C, Holmstrup M. 2009a. Impacts of heavy metals, polyaromatic hydrocarbons, and pesticides on freeze tolerance of the earthworm *Dendrobaena octaedra*. *Environ Toxicol Chem* 28:2341-2347.

Bindesbøl A-M, Bayley M, Damgaard C, Hedlund K, Holmstrup, M. 2009b. Changes in membrane phospholipids as a mechanistic explanation for decreased freeze tolerance in earthworms exposed to sublethal copper concentrations. *Environ Sci Technol* 43:5495-5500.

Bindesbøl A, Holmstrup M, Damgaard CF, Bayley M. 2005. Stress synergy between environmentally realistic levels of copper and frost in the earthworm *Dendrobaena octaedra*. *Environ Toxicol Chem* 24:1462-1467.

Blakely JK, Neher DA, Spongberg AL. 2002. Soil invertebrate and microbial communities, and decomposition as indicators of polycyclic aromatic hydrocarbon contamination. *Appl Soil Ecol* 21:71-88.

Bohlen PJ, Scheu S, Hale CM, McLean MA, Groffman PM, Parkinson D. 2004a. Non-native invasive earthworms as agents of change in northern temperate forests. *Front Ecol Environ* 2:247-435.

Bohlen PJ, Groffman PM, Fahey TJ, Fisk MC, Suárez E, Pelletier DM, Fahey RT. 2004b. Ecosystem consequences of exotic earthworm invasion of north temperate forests. *Ecosystems* 7:1-12.

Boutin C, Elmegaard N, Kjær C. 2004. Toxicity Testing of Fifteen Non-Crop Plant Species with Six Herbicides in a Greenhouse Experiment: Implications for Risk Assessment. *Ecotoxicol* 13:349-369.

Boutin C, White AL, Carpenter D. 2010. Measuring variability in phytotoxicity testing using crop and wild plant species. *Environ Toxicol Chem* 29:327-337.

Braida WJ, White JC, Ferrandino FJ, Pignatello JJ. 2001. Effect of solute concentration on sorption of polyaromatic hydrocarbons in soil: uptake rates. *Environ Sci Technol* 35:2765-2772.

Brown PJ, Long SM, Spurgeon DJ, Svendsen C, Hankard PK. 2004. Toxicological and biochemical responses of the earthworm *Lumbricus rubellus* to pyrene, a non-carcinogenic polycyclic aromatic hydrocarbon. *Chemosphere* 57:1675-1681.

Brusseau ML, Jessup RE, Rao PSC. 1991. Nonequilibrium sorption of organic chemicals: elucidation of rate-limiting processes. *Environ Sci Technol* 25:134-142.

Cameron EK, Bayne EM, Clapperton MJ. 2007. Human-facilitated invasion of exotic earthworms in northern boreal forests. *J Appl Ecol* 14:482-490.

Canadian Council of Ministers of the Environment. 2007. Canadian soil quality guidelines for the protection of environmental and human health: Summary tables. Updated September, 2007. In: *Canadian Environmental Quality Guidelines*, 1999, Canadian Council of Ministers of the Environment, Winnipeg, Manitoba, Canada.

Canadian Council of Ministers of the Environment. 2008. *Canada-Wide Standard for Petroleum Hydrocarbons (PHC) in Soil: Scientific Rationale*. Supporting Technical Document PN 1399. Winnipeg, Manitoba, Canada.

Cannon RJC, Block W. 1988. Cold tolerance of microarthropods. *Biol Rev Camb Philos* 63:23-77.

Carmichael LM, Pfaender FK. 1997. Polynuclear aromatic hydrocarbon metabolism in soils: relationship to soil characteristics and preexposure. *Environ Toxicol Chem* 16:666-675.

Carmo AM, Hundal LS, Thompson ML. 2000. Sorption of hydrophobic organic compounds by soil materials: application of unit equivalent Freundlich coefficients. *Environ Sci Technol* 34:4363-4369.

Cermak JH, Stephenson GL, Birkholz D, Wang Z, Dixon DG. 2010. Toxicity of petroleum hydrocarbon distillates to soils organisms. *Environ Toxicol Chem* 29:2685-2694.

Chapman EEV, Dave G, Murimboh JD. 2013. A review of metal (Pb and Zn) sensitive and pH tolerant bioassay organisms for risk screening of metal-contaminated acidic soils. *Environ Pollut* 179:326-342.

Christiansen K, Bellinger P. 1998. The Collembola of North America North of the Rio Grande. A Taxonomic Analysis. Grinnell College, Grinnell, IA.

Clapperton MJ, Miller JJ, Larney FJ, Lindwall CW. 1997. Earthworm populations as affected by long-term tillage practices in southern Alberta, Canada. *Soil Biol Biochem* 29:631-633.

Coleman DC, Crossley DA Jr, Hendrix PF. 2004. *Fundamentals of Soil Ecology*. Academic, London, UK.

Convey P. Seasonal lipid contents of Antarctic microarthropods. *Exp Appl Acarol* 15:219-231.

Cortet J, Dillon D, Joffre R, Ourcival N, Poinsot-Balaguer N. 2002. Effects of pesticides on organic matter recycling and microarthropods in a maize field: use and discussion of the litterbag methodology. *Euro J Soil Biol* 38:261-265.

Cortet J, Joffre R, Elmholt S, Coeurdassier M, Scheifler R, Krogh PH. 2006. Interspecific relationships among soil invertebrates influence pollutant effects of phenanthrene. *Environ Toxicol Chem* 25:120-127.

Crampton AL, Baxter GD, Barker SC. 1999. A new family of cytochrome P450 genes (*CYP41*) from the cattle tick, *Boophilus microplus*. *Insect Biochem Molec* 29:829-834.

Crommentuijn T, Doodeman CJAM, Doornekamp A, van der Pol JJC , Bedaux JJM, van Gestel CAM. 1994. Lethal body concentrations and accumulation patterns determine time-dependent toxicity of cadmium in soil arthropods. *Environ Toxicol Chem* 13:1781-1789.

Crommentuijn T, Doodeman CJAM, van der Pol JJC, Doornekamp A, Rademaker MCJ, van Gestel CAM. 1995. Sublethal sensitivity index as an ecotoxicity parameter measuring energy allocation under toxicant stress: application to cadmium in soil arthropods. *Ecotox Environ Safe* 31:192-200.

Croser C, Renault S, Franklin J, Zwiazek J. 2001. The effect of salinity on the emergence and seedling growth of *Picea mariana*, *Picea glauca* and *Pinus banksiana*. *Environ Pollut* 115:9-16.

Crossley DA Jr, Bohnsack KK. 1960. Long-term ecological study in the Oak Ridge area: III. The oribatid mite fauna in pine litter. *Ecology* 41:628-638.

Denneman CAJ , van Straalen NM. 1991. The toxicity of lead and copper in reproduction tests using the oribatid mite *Platynothrus peltifer*. *Pedobiologia* 35:305-311.

Dodd M, Addison J. 2010. Toxicity of methyl tert butyl ether to soil invertebrates (springtails: *Folsomia candida*, *Proisotoma minuta*, and *Onychiurus folsomi*) and lettuce (*Lactuca sativa*). *Environ Toxicol Chem* 29:338-346.

Domene X, Chelinho S, Sousa JP. 2010. Effects of nonylphenol on a soil community using microcosms. *J Soil Sediment* 10:556-567.

Dorn PB, Salanitro JP, Wisniewski HL. 1998. Assessment of the acute toxicity of crude oils in soils using earthworms, microtox, and plants. *Chemosphere* 37: 845-860.

Droge ST, Paumen ML, Bleeker EA, Kraak MH, van Gestel CAM. 2006. Chronic toxicity of polycyclic aromatic compounds to the springtail *Folsomia candida* and the enchytraeid *Enchytraeus crypticus*. *Environ Toxicol Chem* 25:2423-2431.

Dymond P, Scheu S, Parkinson D. 1997. Density and distribution of *Dendrobaena octaedra* (Lumbricidae) in aspen and pine forests in the Canadian Rocky Mountains (Alberta). *Soil Biol Biochem* 29:265-273.

EcoDynamics Consulting Ltd. 2007. *Sampling of Contaminated Soils in the Swan Hills and Peace River Areas of Alberta*. Prepared for Environment Canada, Ottawa, Ontario, Canada.

EcoDynamics Consulting Ltd. 2008. *Sampling of a Reference Soil in the Bathurst Region of New Brunswick - Ecological Site Characterization*. Prepared for Environment Canada, Ottawa, Ontario, Canada.

EcoDynamics Consulting Ltd. 2011. Ecological *Description of the Ontario Reference Site*. Prepared for Environment Canada, Ottawa, Ontario, Canada.

Edwards CA, Bohlen PJ. 1996. *Biology and Ecology of Earthworms*. Chapman and Hall, London.

Eisenhauer N, Partsch S, Parkinson D, Scheu S. 2007. Invasion of a deciduous forest by earthworms: Changes in soil chemistry, microflora, microarthropods and vegetation. *Soil Biol Biochem* 39:1099–1110.

Environment Canada. 2003. Toxicity test methodologies for assessing the impacts of contaminant mixtures in soil using terrestrial species of ecological relevance to Canadian soil systems. Workshop Proceedings, February 19-21, 2003. Environment Canada's Pacific Environmental Sciences Centre, Vancouver, British Colombia, Canada.

Environment Canada. 2004. *Biological Test Method: Tests for Toxicity of Contaminated Soils to Earthworms (*Eisenia andrei, Eisenia fetida, *or* Lumbricus terrestris*).* EPS 1/RM/43. Environmental Protection Series, Environment Canada, Ottawa, Ontario, Canada.

Environment Canada. 2005a. *Guidance Document: Statistical Methods for Environmental Toxicity Tests*. EPS 1/RM/46. Environmental Protection Series, Ottawa, ON.

Environment Canada. 2005b. *Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil*. EPS 1/RM/45. Environmental Protection Series, Environment Canada, Ottawa, Ontario, Canada.

Environment Canada. 2007. *Biological Test Method: Test for Measuring Survival and Reproduction of Springtails Exposed to Contaminants in Soil*. EPS 1/RM/47. Environmental Protection Series, Ottawa, ON.

Environment Canada. 2010. *Development and Standardization of New Toxicity Test Methodologies and Guidance for Assessment and Remediation of Impacts from Hydrocarbon and Brine Contamination on Boreal Forest, Taiga and Northern Soil using Organisms Representative of the Ecozone Regions*. Five Year Technical Progress Report. Environment Canada, Ottawa, Ontario, Canada.

Erstfeld KM, Snow-Ashbrook J. 1999. Effects of chronic low-level PAH contamination of soil invertebrate communities. *Chemosphere* 39:2117-2139.

Feisthauer NC, Stephenson GL, Princz JI, Scroggins RP. 2006. Effects of metal-contaminated forest soils from the Canadian shield to terrestrial organisms. *Environ Toxicol Chem* 25:823-835.

Fernández MD, Vega MM, Tarazona JV. 2006. Risk-based ecological soil quality criteria for the characterization of contaminated soils. Combination of chemical and biological tools. *Sci Total Environ* 366:466–484.

Fischer E, Molnár L. 1997. Growth and reproduction of *Eisenia fetida* (Oligochaeta, Lumbricidae) in semi-natural soil containing various metal chlorides. *Soil Biol Biochem* 29:667-670.

Fisher, RF, Binkely D. 2000. *Ecology and Management of Forest Soils*, 3rd edition. John Wiley & Sons, Inc. New York, New York, USA.

Franklin JA, Renault S, Croser C, Zwiazek JJ, MacKinnon M. 2002. Jack pine growth and elemental composition are affected by saline tailings water. *J Environ Qual* 31:648-653.

Gates GE. 1982. Farewell to North American megadriles. *Megadrilogica* 4:12-77.

Gergócs V, Hufnagel L. 2009. Application of oribatid mites as indicators: a review. *Appl Ecol Environ Res* 7:79-98.

Goksøyr A, Förlin L. 1992. The cytochrome *P*-450 system in fish, aquatic toxicology and environmental monitoring. *Aquat Toxicol* 22: 287–312.

Greensdale P, Vaughan GT. 2003. A comparison of Collembola species for toxicity testing of Australian soils. *Pedobiologia* 47:171-179.

Hågvar S, Abrahamsen G. 1980. Colonisation by Enchytraeidae, Collembola and Acari in sterile soil samples with adjusted pH levels. *Oikos* 34:245-258.

Hågvar S, Abrahamsen G. 1990. Microarthropoda and enchytraeidae (Oligochaeta) in a naturally lead-contaminated soil: a gradient study. *Environ Entomol* 19:1263-1277.

Hall AT, Oris JT. 1991. Anthracene reduces reproductive potential and is maternally transferred during long-term exposure in fathead minnows. *Aquat Toxicol* 19:249-264.

Hansen RA. 1999. Red oak litter promotes a microarthropod functional group that accelerates its decomposition. *Plant Soil* 209:37-45.

Hansen RA. 2000. Effects of habitat complexity and composition on a diverse litter microarthropod assemblage. *Ecology*, 81(4):1120-1132.

Heckmann L-H, Maraldo K, Krogh PH. 2005. Life stage specific impact of Dimethoate on the predatory mite *Hypoaspis aculeifer* Canestrini (Gamasida: Laelapidae). *Environ Sci Technol* 39: 7154-7157.

Heethoff M, Laumann M, Bergmann P. 2007. Adding to the Reproductive Biology of the Parthenogenetic Oribatid Mite, *Archegozetes longisetosus* (Acari, Oribatida, Trhypochthoniidae). *Turk J Zool* 31:151-159.

Heneghan L, Coleman DC, Zou X, Crossley DA Jr, Haine BL. 1999. Soil microarthropod contributions to decomposition dynamics: tropical-temperate comparisons of a single substrate. *Ecology* 80:1873-1882.

Hofman J, Rhodes A, Semple KT. 2008. Fate and behaviour of phenanthrene in the natural and artificial soils. *Environ Pollut* 152:468-475.

Holmstrup M, Sørensen LI, Bindesbøl A, Hedlund K. 2007. Cold acclimation and lipid composition in the earthworm *Dendrobaena octaedra*. *Comp Biochem Physiol A Mol Integr Physiol* 147:911-919.

Hopkin SP. 1997. *Biology of Springtails (Insecta: Collembola).* Oxford University Press Inc., New York, New York, USA.

Howat DR. 2000. *Acceptable Salinity, Sodicity and pH Values for Boreal Forest Reclamation*. Alberta Environment, Environmental Sciences Division Rep. ESD/LM/00-2.

Hubert J, Žilová M, Pekár S. 2001. Feeding preferences and gut contents of three panphytophagous oribatid mites (Acari: Oribatida). *Eur J Soil Biol* 37:197-208.

Hutchinson TC, Whitby LM. 1977. The effects of acid rainfall and heavy metal particulates on a boreal forest ecosystem near the Sudbury smelting region of Canada. *Water Air Soil Poll* 7:421-438.

International Organization for Standardization (ISO). 1993. *Soil quality – Determination of the effects of pollutants on soil flora. Part 1: Method for the measurement of inhibition of root growth*. ISO 11269-1. Geneva, Switzerland.

International Organization for Standardization (ISO). 1995. *Soil Quality – Determination of the Effects of Pollutants on Soil Flora. Part 2: Effects of Chemicals on the Emergence and Growth of Higher Plants*. ISO 11269-2, Geneva, Switzerland.

International Organization for Standardization (ISO). 1998. *Soil quality – Effects of pollutants on earthworms (*Eisenia fetida*). Part 2: Determination of effects on reproduction*. ISO 11269-2. Geneva, Switzerland.

International Organization for Standardization (ISO). 1999. *Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants*. ISO 11267. Geneva, Switzerland.

Jager T, Sánchez FAA, Muijs B, van der Velde EG, Posthuma L. 2000. Toxicokinetics of polycyclic aromatic hydrocarbons in *Eisenia andrei* (Oligochaeta) using spiked soil. *Environ Toxicol Chem* 19:953-961.

Janssen MPM, Hogervorst RF. 1993. Metal accumulation in soil arthropods in relation to micro-nutrients. *Environ Pollut* 79:181-89.

Johnston JM, Crossley DA Jr. 2002. Forest ecosystem recovery in the southeast US: soil ecology as an essential component of ecosystem management. *Forest Ecol Manag* 155:187-203.

Jones OAH, Spurgeon DJ, Svendsen C, Griffin JL. 2008. A metabolomics based approach to assessing the toxicity of the polyaromatic hydrocarbon pyrene to the earthworm *Lumbricus rubellus*. *Chemosphere* 71:601-609.

Kan AT, Fu G, Tomson MB. 1994. Adsorption/desorption hysteresis in organic pollutant and soil/sediment interaction. *Environ Sci Technol* 28:859-867.

Kaputska, LA. 1997. Selection of Phytotoxicity Tests for Use in Ecological Risk Assessments, p. 515–548, In, Wang W, Gorsuch JW, Hughes JS, eds, *Plants for Environmental Studies*. CRC Press, New York, NY, pp. 515-548.

Kelsey JW, Alexander M. 1997. Declining bioavailability and inappropriate estimation of risk of persistent compounds. *Environ Toxicol Chem* 16:582-585.

Kelsey JW, Kottler BD, Alexander M. 1997. Selective chemical extractants to predict bioavailability of soil-aged organic chemicals. *Environ Sci Technol* 31:214-217.

Khalil MA, Janssen TKS, Berg MP, van Straalen NM. 2009. Identification of metal-responsive oribatid mites in a comparative survey of polluted soils. *Pedobiologia* 52:207-221.

Khan AG, Kuek C, Chaudry TM, Khoo CS, Hayes WJ. 2000. Role of plants, mycorrhizae, and phytochelators in heavy metal contaminated land remediation. *Chemosphere* 41:197-207.

Khasa PD, Hambling B, Kernaghan G, Fung M, Ngimbi E. 2002. Genetic variability in salt tolerance of selected boreal woody seedlings. *Forest Ecol Manage* 165:257-269.

Khasa DP, Fung M, Logan B. 2005. Early growth response of container-grown selected woody boreal seedlings in amended composite tailings and tailings sand. *Bioresource Technol* 96:857-864.

Kirkey FM, Matthews J, Ryser P. 2012. Metal resistance in populations of red maple (*Acer rubrum L.*) and white birch (*Betula papyrifera Marsh.*) from a metal-contaminated region and neighbouring non-contaminated regions. *Environ Pollut* 164:53-58.

Klironomos JN, Kendrick B. 1995. Relationships among microarthropods, fungi, and their environment. *Plant Soil* 170:183-197.

Köhler HR, Alberti G, Seniczak S, Seniczak A. 2005. Lead-induced hsp70 and hsp60 pattern transformation and leg malformation during postembryonic development in the oribatid mite, *Archegozetes longisetosus* Aoki. *Comp Biochem Phys C* 141:398-405.

Koukol O, Mourek J, Janovský Z, Černá K. 2009. Do oribatid mites (Acari: Oribatida) show a higher preference for ubiquitous vs. specialized saprotrophic fungi from pine litter? *Soil Biol Biochem* 41:1124-1131.

Krantz GW, Walter DE (Ed.). 2009. *A Manual of Acarology*, 3rd edition. Texas Tech University Press, Lubbock, TX, USA.

Krauss M, Wilcke W, Zech W. 2000. Availability of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) to earthworms in urban soils. *Environ Sci Technol* 34:4335-4340.

Kula H, Heimbach U. 1998. Reproducibility in soil toxicity testing. *In*: H. Løkke and CAM van Gestel (Ed.), *Handbook of Soil Invertebrate Toxicity Tests*. John Wiley & Sons, Chichester, UK, pp 33-40.

Landrum PF. 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod *Pontoporeia hoyi*. *Environ Sci Technol* 23:588-595.

Langdon CJ, Piearce TG, Meharg AA, Semple KT. 2001. Resistance to copper toxicity in populations of the earthworms *Lumbricus rubellus* and *Dendrodrilus rubidus* from contaminated mine wastes. *Environ Toxicol Chem* 20:2336-2341.

Lavkulich LM, Arocena JM. 2011. Luvisolic soils of Canada: genesis, distribution, and classification. *Can J Soil Sci* 91:781-806.

Lebrun P, van Straalen NM. 1995. Oribatid mites: prospects for their use in ecotoxicology. *Exp Appl Acarol* 19:361-379.

Leskiw LA, Sedor RB, Welsh CM, Zeleke TB. 2012. Soil and vegetation recovery after a well blowout and salt water release in northeastern British Columbia. *Can J Soil Sci* 92:179-190.

Letang DL, de Groot WJ. 2012. Forest floor depths and fuel loads in upland Canadian forests. *Can J For Res* 42:1551-1565.

Lilles EB, Purdy BG, Macdonald SE, Chang SX. 2012. Growth of aspen and white spruce on naturally saline sites in northern Alberta: Implications for development of boreal forest vegetation on reclaimed saline soils. *Can J Soil Sci* 92:213-227.

Ludwig M, Kratzmann M, Alberti G. 1992. Observations on the proventricular glands ('organes racdmiformes') of the oribatid mite *Chamobates borealis* (Acari, Oribatida): an organ of interest for studies on adaptation of animals to acid soils. *Exp Appl Acarol* 15:49-57.

Luxton M. 1981. Studies on the Oribatid mites of a Danish Beech wood soil IV. Developmental biology. *Pedobiologia*, 21: 312–340.

Luz TN da, Ribeiro R, Sousa JP. 2004. Avoidance tests with Collembola and earthworms as early screening tools for site-specific assessment of polluted soils. *Environ Toxicol Chem* 23:2188-2193.

Ma W-C, Immerzeel J, Bodt J. 1995. Earthworm and food interactions on bioaccumulation and disappearance in soil of polycyclic aromatic hydrocarbons: studies on phenanthrene and fluoranthene. *Ecotox Environ Safe* 32:226-232.

Maraun M, Alphei J, Bonkowski M, Buryn R, Migge S, Peter M, Schaefer M, Scheu S. 1999. Middens of the earthworm *L. terrestris* (Lumbricidae): microhabitats for micro- and Mesofauna in forest soil. *Pedobiologia* 43:276-287.

Maraun M, Scheu S. 2000. The structure of oribatid mite communities (Acari, Oribatida): patterns, mechanisms and implications for future research. *Ecography* 23:374-383.

Marinissen JCY, van den Bosch, F. 1992. Colonization of new habitats by earthworms. *Oecologia* 91:371-376 .

Massoud Z, Betsch-Pinot MC. 1974. Observations sur la ponte de *Proisotoma minuta* (Collembola Isotomidae). *Pedobiologia BD* 14 :208-212.

Mazurová E, Hilscherová K, Jálová V, Köhler HR, Triebskornc R, Giesy JP, Bláha L. 2008. Endocrine effects of contaminated sediments on the freshwater snail *Potamopyrgus antipodarum in vivo* and in the cell bioassays *in vitro*. *Aquat Toxicol* 89:172-179.

McLean MA, Parkinson D. 1997. Changes in structure, organic matter and microbial activity in pine forest soil following the introduction of *Dendrobaena octaedra* (Oligochaeta, Lumbricidae). *Soil Biol Biochem* 29:537-540.

McLean MA, Parkinson D. 2000. Introduction of the epigeic earthworm *Dendrobaena octaedra* changes the oribatid community and microarthropod abundances in a pine forest. *Soil Biol Biochem* 32:1671-1681.

Michereff-Filho M, Guedes RNC, Della-Lucia TMC, Michereff MFF, Cruz I. 2004. Non-target impact of chlorpyrifos on soil arthropods associated with no-tillage corn fields in Brazil. *Int J Pest Manag* 50:91-99.

Mihajlovich M, Pitt D, Blake P. 2004. Comparison of four glyphosate herbicide formulations for white spruce release treatment. *Forest Chron* 80:608-611.

Minor MA, Norton RA. 2004. Effects of soil amendments on assemblages of soil mites (Acari: Oribatida, Mesostigmata) in short-rotation willow plantings in central New York. *Can J Forest Res* 34:1417-1425.

Mitchell MJ. 1977. Population dynamics of oribatid mites (Acari, Cryptostigmata) in as aspen woodland soil. *Pedobiologia*, 17:305-319.

Moore JC, Walter DE, Hunt HW. 1988. Arthropod regulation of micro- and mesobiota in below-ground detrital food webs. *Annu Rev Entomol*, 33:419-439.

Moore JD, Ouimet R, Reynolds JW. 2009. Premières mentions de vers de terre dans trois écosystèmes forestiers du Bouclier canadien. *Nat Can* 133:31-37.

Morgan AJ, Turner MP. 2005. Quantitative ultrastructure of metal-sequestering cells reflects intersite and interspecies differences in earthworm metal burdens. *Arch Environ Cont Tox* 49:45-52.

Mulder H, Breure AM, van Andel JG, Grotenhius JTC, Rulkens WH. 2000. Effect of mass-transfer limitations on bioavailability of sorbed naphthalene in synthetic model soil matrices. *Environ Toxicol Chem* 19:2224-2234.

Norberg-King TJ. 1993. *A Linear Interpolation Method for Sublethal Toxicity: the Inhibition Concentration (ICp) Approach (Ver 2.0)*. Technical Report 03-93. National Effluent Toxicity Assessment Center, U.S. Environmental Protection Agency, Duluth, MN.

Norton RA. 1985. Aspects of the biology and systematics of soil arachnids, particularly saprophagous and mycophagous mites. *Quaest Entomol*, 21:523-541.

Norton, RA. 1994. Evolutionary aspects of oribatid mites life histories and consequences for the origin of the Astigmata. *In*: Houck MA (Ed.), *Mites: Ecological and Evolutionary Analyses of Life-History Patterns*, Chapman & Hall, New York, pp. 99-135.

Norton RA, Bonamo PM, Grierson JD, Shear WA. 1988. Oribatid mite fossils from a terrestrial Devonian deposit near Gilboa, New York. *J Paleontol* 62:259-69.

Norton RA, Behan-Pelletier VM. 1991. Calcium carbonate and calcium oxalate as cuticular hardening agents in oribatid mites (Acari: Oribatida). *Can J Zoolog*, 69: 1504−1511.

Norton RA, Behan-Pelletier VM. 2006. *Soil Acarology: Oribatid Mites*. 56th Annual Acarology Summer Program, July 10-15, 2006.

Norton RA, Behan-Pelletier V M. 2009. Oribatida. *In*: Krantz GW and Walter DE (Ed.), *A Manual of Acarology*, 3rd edition. Texas Tech University Press, Lubbock, TX, USA, pp 430-564.

Nursita AI, Singh B, Lees E. 2005. The effects of cadmium, copper, lead, and zinc on the growth and reproduction of *Proisotoma minuta* Tullberg (Collembola). *Ecotox Environ Safe* 60:306-314.

Organization for Economic Cooperation and Development (OECD). 1984. *Test No: 208: Terrestrial Plants, Growth Test, Test No. 208*. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, France.

Organization for Economic Cooperation and Development (OECD). 2008. *Test No. 226: Predatory Mite (Hypoaspis (Geolaelaps) aculeifer) Reproduction Test in Soil*. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, France.

Organization for Economic Cooperation and Development (OECD). 2009. *Test No. 232: Collembolan Reproduction Test in Soil*. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, France.

Organization for Economic Cooperation and Development (OECD). 2010. *Test No. 317: Bioaccumulation in Terrestrial Oligochaetes*. OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, France.

Ohkubo N. 2001. A Revision of Oppiidae and its Allies (Acarina: Oribatida) of Japan 1. Genus Lasiobelba. *Journal of the Acarological Society of Japan* 10:97-109.

Oliveira AR, Castro TR, Capalbo DMF, Delalibera I Jr. 2007. Toxicological evaluation of genetically modiWed cotton (Bollgard®) and Dipel® WP on the non-target soil mite *Scheloribates praeincisus* (Acari: Oribatida). *Exp Appl Acarol* 41:191–201.

Olszyk D, Pfleeger T, Lee EH, Burdick C, King G, Plocher M, Kern J. 2008. Selecting and Evaluating Native Plants for Region-Specific Phytotoxicity Testing. *Integrated Environ Assess Manag* 4:105-117.

Osler GHR, Westhorpe D, Oliver I. 2001. The short-term effects of endosulfan discharges on eucalypt floodplain soil microarthropods. *Appl Soil Ecol* 16:263-273.

Overgaard J, Slotsbo S, Holmstrup M, Bayley M. 2007. Determining factors for cryoprotectant accumulation in the freeze-tolerant earthworm, *Dendrobaena octaedra*. *J Exp Zool A* 307:578-589.

Owojori OJ, Healey J, Princz J, Siciliano SD. 2011. Can avoidance behaviour of the mite *Oppia nitens* be used as a rapid toxicity test for soils contaminated with metals or organic chemicals? *Environ Toxicol Chem* 11:2594-2601.

Owojori OJ, Reinecke AJ, Voua-Otomo P, Reinecke SA. 2009. Comparative study of the effects of salinity on life-cycle parameters of four soil-dwelling species (*Folsomia candida, Enchytraeus doerjesi, Eisenia fetida* and *Aporrectodea caliginosa*). *Pedobiologia* 52:351-360.

Owojori OJ, Siciliano S. 2012. Accumulation and toxicity of metals (copper, zinc, cadmium, and lead) and organic compounds (geraniol and benzo[a]pyrene) in the oribatid mite *Oppia nitens*. *Environ Toxicol Chem* 31:1639-1648.

Owojori OJ, Waszak K, Römbke J. 2014. Avoidance and reproduction tests with the predatory mite *Hypoaspis aculeifer*: effects of different chemical substances. *Environ Toxicol Chem* 33:230-237.

Parmelee RW, Wentsel RS, Phillips CT, Simini M, Checkai RT. 1993. Soil microcosm for testing the effects of chemical pollutants on soil fauna communities and trophic structure. *Environ Toxicol Chem* 12:1477–1486.

Park E-K. 2007. Effect of laboratory culture conditions on population growth of *Proisotoma minuta* (Tullberg) (Collembola: Isotomidae). *Entomol Sci* 10**:**135-140.

Park E-K, Lees EM. 2004. The interaction of endosulfan with the Collembolan, *Proisotoma minuta* (Tullberg): toxicity, the effects of sub-lethal concentrations and metabolism. *Pest Manag Sci* 60:710-718.

Park E-K, Lees EM. 2006. Determination of Oxidative Metabolism in Collembolan *Proisotoma minuta* (Tullberg). *J Environ Sci Heal B* 41:57-65.

Parsons WFJ, Parkinson D. 1986. Species composition, distribution and abundance of Collembolan colonizing reclaimed mine spoil in Alberta. *Pedobiologia* 29:33-45.

Pennock D, Bedard-Haughn A, Viaud V. 2011. Chernozemic soils of Canada: genesis, distribution and classification. *Can J Soil Sci* 91:719-747.

Pottelberge SV, van Leeuwen T, van Amermaet K, Tirry L. 2008. Induction of cytochrome P450 monooxygenase activity in the two-spotted spider mite *Tetranychus urticae* and its influence on acaricide toxicity. *Pestic Biochem Phys* 91:128-133.

Princz JI, Behan-Pelletier VM, Scroggins RP, Siciliano SD. 2010. Oribatid mites in soil toxicity testing – the use of *Oppia nitens* (C.L. Koch) as a new test species. *Environ Toxicol Chem* 29:971-979.

Princz JI, Moody M, Fraser C, van der Vliet L, Lemieux H, Scroggins R, Siciliano SD. 2012. Evaluation of a new battery of toxicity tests for boreal forest soil: assessment of the impact of hydrocarbons and salts. *Environ Toxicol Chem* 31:766-777.

Prinzing A, Kretzlera S, Badejo A, Beck L. 2002. Traits of oribatid mite species that tolerate habitat disturbance due to pesticide application. *Soil Biol Biochem* 34:1655-1661.

Proctor H. 1998. *Acariformes: the "mite-like" mites*. Version 09 August 1998. *http://tolweb.org/Acariformes/2563/1998.08.09. In* The Tree of Life Web Project, *http://tolweb.org/*

Raspotnig G, Leis, H-J. 2009. Wearing a raincoat: exocrine secretions contain anti-wetting agents in the oribatid mite, *Liacarus subterraneus* (Acari: Oribatida). *Exp Appl Acarol* 47:179-190.

Renault S, Zwiazek JJ, Fung M, Tuttle S. 2000. Germination, growth and gas exchange of selected boreal forest seedlings in soil containing oil sands tailings. *Environ Pollut* 107:357-365.

Rentz JA, Chapman B, Alvarez PJJ, Schnoor JL. 2003. Stimulation of hybrid poplar growth in petroleum-contaminated soils through oxygen addition and soil nutrient amendments. *Int J Phytoremediat* 5:57-72.

Reynolds JW. 1977. *The earthworms (Lumbricidae and Sparganophilidae) of Ontario*. Royal Ontario Museum, Ontario, Canada.

Robertson BK, Alexander M. 1998. Sequestration of DDT and dieldrin in soil: disappearance of acute toxicity but not the compounds. *Environ Toxicol Chem* 17:1034-1038.

Robertson SJ, McGill WB, Massicotte HB, Rutherford PM. 2007. Petroleum hydrocarbon contamination in boreal forest soils: a mycorrhizal ecosystems perspective. *Biol Rev* 82:213-240.

Römbke J, Jänsch S, Scroggins R. 2006. Identification of potential organisms of relevance to Canadian boreal forest and northern lands for testing of contaminated soils. *Environ Rev* 14:137-167.

Roth M. 1993. Investigations on lead in the soil invertebrates of a forest ecosystem. *Pedobiologia* 37:270-279.

Rundgren S, Nilsson P. 1997. Sublethal effects of aluminium on earthworms in acid soil: the usefulness of *Dendrodrilus rubidus* (Sav.) in a laboratory test system. *Pedobiologia* 41:417-436.

Salanitro JP, Dorn PB, Huesemann MH, Moore KO, Rhodes IA, Rice Jackson LM, Vipond TE, Western MM, Wisniewski HL. 1997. Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment. *Environ Sci Technol* 31:1769-1776.

Salminen J, Haimi J. 1997. Effects of pentachlorophenol on soil organisms and decomposition in forest soil. *J Appl Ecol* 34:101-110.

Sanborn P, Lamontagne L, Hendershot W. 2011. Podzolic soils of Canada: genesis, distribution and classification. *Can J Soil Sci* 91:843-880.

Sanderson LA, McLaughlin JA, Antunes PM. 2012. The last great forest: a review of the status of invasive species in the North American boreal forest. *Forestry* 85:329-339.

Santorufo L, Carotenuto R, Rocco A, De Luca Picione F, Maisto G. 2012. *Orthonychiurus pseudostachianus* (Collembola) as a toxicity test organism and selection of an ecotoxicological test battery to assess soil quality. *Appl Soil Ecol* 54:49-54.

Saskatchewan Research Council. 2003. *Boreal Forest Plant Species for Testing of Contaminated Soil*. Publication No. 11627-2E03. Saskatchewan Research Council, Saskatoon, Saskatchewan, Canada.

Saterbak A, Toy RJ, Wong DCL, McMain BJ, Williams MP, Dorn PB, Brzuzy LP, Chai EY, Salanitro JP. 1999. Ecotoxicological and Analytical Assessment of Hydrocarbon-contaminated Soils and Application to Ecological Risk Assessment. *Environ Toxicol Chem* 18:1591-1607.

Schneider K, Maraun M. 2005. Feeding preferences among dark pigmented fungal taxa (‘‘Dematiacea’’) indicate limited trophic niche differentiation of oribatid mites (Oribatida, Acari). *Pedobiologia* 49:61-67.

Schmidt SN, Smith KE, Holmstrup M, Mayer P. 2013. Uptake and toxicity of polycyclic aromatic hydrocarbons in terrestrial springtails - studying bioconcentration kinetics and linking toxicity to chemical activity. *Environ Toxicol Chem* 32:361-369.

Seastedt TR. 1984. The role of microarthropods in decomposition and mineralization processes. *Ann Rev Entomol* 29:25-46.

Sengbusch HG, Sengbusch CH. 1970. Post-embryonic development of *Oppia nitens* (Acarina: Oribatei). *J N Y Entomol Soc* 78:207-214.

Seniczak S. 1975a. Morphology of juvenile stages of some Oppiidae (Acarina, Oribatei) II. *Pedobiologia* 15:262-275.

Seniczak S. 1975b. Morphology of juvenile stages of some Oppiidae (Acarina, Oribatei) I. *Pedobiologia* 15:249-261.

Seniczak A. 2006. The effect of density on life-history parameters and morphology of *Archegozetes longisetosus* Aoki, 1965 (Acari: Oribatida) in laboratory conditions. *Biological Letters* 43:209-213.

Seniczak A, Ligocka A, Seniczak S, Paluszak Z. 2009. The influence of cadmium on life-history parameters and gut microflora of *Archegozetes longisetosus* (Acari: Oribatida) under laboratory conditions. *Exp Appl Acarol* 47:191-200.

Seniczak A, Seniczak S. 2002. The effect of cadmium on *Archegozetes longisetosus* Acari, Oribatida) in laboratory conditions. *Eur J Soil Biol* 38:315-317.

Seniczak S, Klimek A, Gackowski G, Kaczmarek S, Zalewski W. 1997. Effect of copper smelting air pollution on the mites (Acari) associated with young scots pine forests polluted by a copper smelting works at Głogów, Poland. II. Soil mites. *Water Air Soil Poll* 97:287–302.

Seniczak, Seniczak W, Kobiersk M. 2006. Long-term effect of cadmium on the oribatid mite *Archegozetes longisetosus* Aoki, 1965 in laboratory conditions. *Biological Letters* 43:237-242.

Seniczak S, Stefaniak O. 1978. The microflora of the alimentary canal of *Oppia nitens* (Acarina, Oribatei). *Pedobiologia* 18:110-119.

Siepel H. 1995. Are some mites more ecologically exposed to pollution with lead than others? *Exp Appl Acarol* 19:391-398.

Siepel H. 1996. The Importance of Unpredictable and Short-Term Environmental Extremes for Biodiversity in Oribatid Mites. *Biodiversity Letters* 3:26-34.

Singh M, Jain KL, Mathur RB, Dogra D. 1996. Laboratory study of food preferences of some cryptostigmatic mites and their contribution in litter degradation and mineralization in soils. *Ann Biol* 12:335-343.

Skubała P, Kafel A. 2004. Oribatid mite communities and metal bioaccumulation in oribatid species (Acari, Oribatida) along the heavy metal gradient in forest ecosystems. *Environ Pollut* 132:51-60.

Skubała P, Kafel A. 2004. Oribatid mite communities and metal bioaccumulation in oribatid species (Acari, Oribatida) along the heavy metal gradient in forest ecosystems. *Environ Pollut* 132:51-60.

Skubała P, Zaleski T. 2012. Heavy metal sensitivity and bioconcentration in oribatid mites (Acari, Oribatida) gradient study in meadow ecosystems. *Sci Total Environ* 414:364-372.

Smit CE, Moser T, Römbke J. 2012. A new OECD test guideline for the predatory soil mite Hypoaspis aculeifer: results of an international ring test. *Ecotoxicol Environ Saf* 82:56-62.

Smith CAS, Webb KT, Kenny E, Anderson A, Kroetsch D. 2011. Brunisolic soils of Canada: genesis, distribution and classification. *Can J Soil Sci* 91:695-717.

Smith PG, Boutin C, Knopper L. 2013. Vanadium pentoxide phytotoxicity: effects of species selection and nutrient concentration. *Arch Environ Contam Toxicol* 64:87-96.Soil Classification Working Group. 1998. *The Canadian System of Soil Classification*. Agric. and Agri-Food Can. Publ. 1646 (Revised) 187 pp. NRC Research Press, Ottawa

Sorenson PT, Quideau SA, MacKenzie MD, Landhäusser SM, Oh SW. Forest floor development and biochemical properties in reconstructed forest soils. *Appl Soil Ecol* 49:139-147.

Stark JD. 1992. Comparison of the impact of a neem seed-kernel extract formulation 'Margosan-O' and chlorpyrifos on non-target invertebrates inhabiting turf grass. *Pestic Sci* 36:293–299.

Stefaniak O, Seniczak S. 1981. The effect of fungal diet on the development of *Oppia nitens* (Acari, Oribatei) on the microflora of its alimentary tract. *Pedobiologia* 21:202-210.

Stephenson, GL. 2003. *Terrestrial Test Methods for Plants and Soil Invertebrates*. Ph.D. Thesis, Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada.

Stephenson GL, Kuperman RG, Linder GL, Visser S. 2002. Toxicity Tests for Assessing Contaminated Soils and Ground Water. In Sunahara GI, Renoux AY, Thellen C, Gaudet CL, Pilon A, eds, *Environmental Analysis of Contaminated Sites, Ecological and Environmental Toxicology Series*. John Wiley & Sons, Chichester, UK, pp 25-43.

Stroomberg GJ, Zappey H, Steen RJCA, van Gestel CAM, Ariese F, Velthorst NH, van Straalen NM. 2004. PAH biotransformation in terrestrial invertebrates - a new phase II metabolite in isopods and springtails. *Comp Biochem Phys C* 138:129-137.

Sugiura K, Ishihara M, Shimauchi T, Harayama S. 1997. Physicochemical properties and biodegradability of crude oil. *Environ Sci Technol* 31:45-51.

Sverdrup LE, de Vaufleury A, Hartnik T, Hagen SB, Loibner AP, Jensen J. 2006. Effects and uptake of polycyclic aromatic compounds in snails (*Helix aspersa*). *Environ Toxicol Chem* 25:1941-1945.

Sverdrup LE, Hagen SB, Krogh PH, van Gestel CAM. 2007. Benzo[a]pyrene shows low toxicity to three species of terrestrial plants, two soil invertebrates, and soil-nitrifying bacteria. *Ecotox Environ Safe* 66:362-368.

Sverdrup LE, Jensen J, Kelley AE, Krogh PH, Stenersen J. 2002a. Effects of eight polycyclic aromatic compounds on the survival and reproduction of *Enchytraeus crypticus* (Oligochaeta, Clitellata*). Environ Toxicol Chem* 21:109-114.

Sverdrup LE, Jensen J, Krogh PH, Stenersen J. 2002b. Studies on the effect of soil aging on the toxicity of pyrene and phenanthrene to a soil-dwelling springtail. *Environ Toxicol Chem* 21:489-492.

Sverdrup LE, Kelley AE, Krogh PH, Nielsen T, Jensen J, Scott-Fordsmand JJ, Stenersen J. 2001. Effects of eight polycyclic aromatic compounds on the survival and reproduction of the springtail *Folsomia fimetaria* L. (Collembola, Isotomidae). *Environ Toxicol Chem* 20: 1332-1338.

Sverdrup LE, Krogh PH, Nielsen T, Kjaer C, Stenersen J. 2003. Toxicity of eight polycyclic aromatic compounds to red clover (*Trifolium pratense*), ryegrass (*Lolium perenne*), and mustard (*Sinapsis alba*). *Chemosphere* 53:993-1003.

Tang J, Carroquino MJ, Robertson BK, Alexander M. 1998. Combined effect of sequestration and bioremediation in reducing the bioavailability of polycyclic aromatic hydrocarbons in soil. *Environ Sci Technol* 32:3586-3590.

Tirello P, Pozzebon A, Cassanelli S, van Leeuwen T, Duso C. 2012. Resistance to acaricides in Italian strains of *Tetranychus urticae*: toxicological and enzymatic assays. *Exp Appl Acarol* 57:53-64.

Tiunov AV, Hale CM, Holdsworth AR, Vsevolodova-Perel TS. 2006. Invasion patterns of Lumbricidae into the previously earthworm-free areas of northeastern Europe and the western Great Lakes region of North America. *Biol Invasions* 8:1223-1234.

Thomas PJ, Carpenter D, Boutin C, Allison JE. 2014. Rare earth elements (REEs): effects on germination and growth of selected crop and native plant species. *Chemosphere* 96:57-66.United States Environmental Protection Agency (US EPA). 1989. *Protocols for Short Term Toxicity Screening of Hazardous Waste Sites*. Report EPA/600/3-88/029, prepared by Greene JC, Bartels CL, Warren-Hicks WJ, Parkhurst BR, Linder GL, Peterson SA, Miller WE. Corvallis Environmental Research Laboratory, Corvallis, Oregon, USA.

van Brummelen TC, van Gestel CAM, Verweij RA. 1996a. Long-term toxicity of five polycyclic aromatic hydrocarbons for the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*. *Environ Toxicol Chem* 15:1199-1210.

van Brummelen TC, Verweij RA, Wedzinga SA, van Gestel CAM. 1996b. Polycyclic aromatic hydrocarbons in earthworms and isopods from contaminated forest soils. *Chemosphere* 32:315-341.

van Gestel CAM. 2012. Soil ecotoxicology: state of the art and future directions. *ZooKeys* 176:275-296.

van Gestel CAM. 1998. Evaluation of the development status of ecotoxicity tests on soil fauna. In Løkke H, van Gestel CAM, eds, *Handbook of Soil Invertebrate Toxicity Tests*. John Wiley & Sons, Chichester, UK, pp 57-68.

van Gestel CAM, Doornekamp A. 1998. Tests on the Oribatid Mite *Platynothrus peltifer*. In Løkke H, van Gestel CAM, eds, *Handbook of Soil Invertebrate Toxicity Tests*. John Wiley & Sons, Chichester, UK, pp 113-130.

van Gestel CAM, Leon CD, van Straalen NN. 1997. Evaluation of soil fauna ecotoxicology tests regarding their use in risk assessment. *In* Tarradellas J, Bitton G, Rossel D (Ed.) *Soil Ecotoxicology*. CRC Press, Boca Raton, U.S.A. pp. 291-317.

van Straalen NM, Butovsky RO, Pokarzhevskii AD, Zaitseva AS, Verhoef SC. 2001. Metal concentrations in soil and invertebrates in the vicinity of metallurgical factory near Tula (Russia). *Pedobiologia* 45: 451-466.

van Straalen NM, Schobben JHM, de Goede RGM. 1989. Population consequences of cadmium toxicity in soil microarthropods. *Ecotox Environ Safe* 17:190-204.

van Straalen NM, Verhoef HA. 1997. The development of a bioindicator system for soil acidity based on arthropod pH preferences. *J Appl Ecol* 34:217-232.

Vlčková K, Hofman J. 2012. A comparison of POPs bioaccumulation in *Eisenia fetida* in natural and artificial soils and the effects of aging. *Environ Pollut* 160:49-56.

Walter DE. 1985. The effects of litter type and elevation on colonization of mixed coniferous litterbags by oribatid mites. *Pedobiologia* 28:383-387.

Walter DE, Krantz G, Lindquist E. 1996. *Acari: the mites*. Version 13 December 1996. *http://tolweb.org/Acari/2554/1996.12.13 in* The Tree of Life Web Project, *http://tolweb.org/*

Walter DE, Proctor HC. 1999. *Mites: Ecology Evolution and Behaviour*. Wallingford, Oxon, UK: CAB International.

Waxman DJ. 1988. Interactions of hepatic cytochromes P-450 with steroid hormones. *Biochem Pharmacol* 37:71-84.

Webb NR. 1989. Observations on the life cycle of *Steganacarus magnus* (Acari: Cryptostigmata). *Pedobiologia* 33:293-299.

Watmough SA, Hutchinson TC. 1997. Metal resistance in red maple (*Acer rubrum*) callus cultures from mine and smelter sites in Canada. *Can J Forest Res* 27:693-700.

White JC, Hunter M, Nam K, Pignatello JJ, Alexander M. 1999. Correlation between biological and physical availabilities of phenanthrene in soils and soil humin in aging experiments. *Environ Toxicol Chem* 18:1720-1727.

White JC, Kelsey JW, Hatzinger PB, Alexander M. 1997. Factors affecting sequestration and bioavailability of phenanthrene in soils. *Environ Toxicol Chem* 16:2040-2045.Witteveen J, Verhoef HA, Letschert JPW. 1987. Osmotic and ionic regulation in marine littoral Collembola at different salinities. *Ecol Entomol* 12:459-469.

Yeardley RB Jr, Lazorchak JM, Gast LC. 1996. The potential of an earthworm avoidance test for evaluation of hazardous waste sites. *Environ Toxicol Chem* 15:1532–1537.

Yu F, Lin YS. 2005. Pollution characteristics of PAHs in the soils of typical urban industrial production areas and their neighboring residential areas. *Ecol Environ* 14:6-9.

Yu L, Berry RE, Croft BA. 1997. Effects of *Bacillus thuringiensis* toxins in transgenic cotton and potato on *Folsomia candida* (Collembola: Isotomidae) and *Oppia nitens* (Acari: Oribatidae). *J Econ Entomol* 90:113-118.

Zaitsev AS, van Straalen NM. 2001. Species diversity and metal accumulation in oribatid mites (Acari, Oribatida) of forests affected by a metallurgical plant. *Pedobiologia* 45, 467–479 (2001).

Zhang W, Song YF, Gong P, Sun TH, Zhouac QX, Liuab M. 2006. Earthworm cytochrome P450 determination and application as a biomarker for diagnosing PAH exposure. *J Environ Monit* 8:963-967.

APPENDIX A

The following provides a detailed description of the soil types and sampling sites used in the assessment of the suitability of the suite of boreal forest species evaluated for their suitability as ecotoxicity test species for boreal soils (Chapters 3 and 5).

A.1 Podzolic Soils

Three Podzolic soils were collected: two from New Brunswick, and one from Ontario. The Ontario soil was collected outside of Pembroke (ON), near Bonnechere Provincial Park. The soil was characterized as a poorly-drained Gleyed Humo-ferric Podzol, underlain by non-calcareous fluvial-lacustrine deposits. The collection site was dominated by coniferous species (e.g., red pine (*Pinus resinosa*), eastern white pine (*Pinus strobus*), white spruce (*Picea glauca*), and black spruce (*Picea mariana*)), although the area was a mixed wood forest containing both coniferous and deciduous (white birch (*Betula papyrifera*), eastern white cedar (*Thuja occidentalis*), red maple (*Acer rubra*) and eastern hemlock (*Tsuga canadensis*)) species. The ground cover consisted of mainly needle and leaf litter, with bunch berry (*Cornus canadensis*), goldthread (*Coptis trifolia*), and oak fern (*Gymnocarpium dryopteris*) (EcoDynamics Consulting Ltd., 2011). Upon collection, the leaf litter (comprising mainly of coniferous needles) was removed, and the following three horizons collected: Ahe (to a depth of 2 cm) (referred ON07-Ahe), Ae (to a depth of 7 cm) (referred to as ON07-Ae), and Bf (to a depth of 20 cm) (referred to as ON07-Bf).

The soil obtained from the first site in New Brunswick was collected from a mixed wood forest, and characterized as an imperfectly drained Gleyed Humo-ferric Podzol. Full soil and vegetation characterizations were not completed for this site. Two horizons were collected: the first consisting of a mixture of the F-H layer with the A horizons (Ahe-Aegj) (referred to as NB07-2009-FH/A) to a depth of 10 cm below the soil surface, and the second being the B horizon (Bf-Bfgj) (referred to as NB07-2009-B) to a depth of approximately 26 cm.

The soil from the second site in New Brunswick was collected from a mixed wood forest, and was classified as an imperfectly drained Gleyed Humo-ferric Podzol, developed on non-calcareous, medium to moderately fine textured basal or lodgement till (EcoDynamics Consulting Ltd., 2008). The mixed wood forest consisted of beech (*Fagus grandfolia*), red maple (*Acer rubrum*), yellow birch (*Betula alleghaniensis*), sugar maple (*Acer saccharum*) and balsam fir (*Abies balsamea*), underlain by an understory of regenerating maple and balsam fir, and beaked hazel (*Corylus cornuta*). Two horizons were collected: the first consisting of a mixture of the F-H and Ahe-Aegj horizons (to a depth of 8-10 cm) (referred to as NB07-2011-FH/A); and the second consisting of Bf horizon (to a depth of 25 cm) (referred to as NB07-2011-B), with some amounts of Bfgj and BCgj due to the variable and wavy nature of the soil horizon boundaries.

A.2 Brunisolic and Luvisolic Soils

Brunisolic and Luvisolic soils were both collected from Saskatchewan. The first location, a Northern Provincial Forest, was located approximately 20 km north of Love (SK), south of the Boreal Transition/Mid-Boreal Upland Ecoregion boundary, and contained mature white spruce (*Picea glauca*) and trembling aspen (*Populus tremuloides*) with an understory of

aspen suckers, rose (*Rosa spp.*), willow (*Salix spp.*), bunchberry (*Cornus canadensis*), and twinflower (*Linnaea borealis*) (EcoDynamics Consulting Ltd., 2007); the soil was characterized as a moderately-well drained Dark Gray Luvisol with a loam to very sandy loam texture. Three horizons were collected from the site: the LFH (10-cm thickness) (referred to as SK05-1-LFH), the Ahe horizon to a depth of approximately 10 cm (referred to as SK05-1-Ahe), and the Bt horizon to a depth of approximately 25 cm (referred to as SK05-1-Bt).

The second location was located in the northwest corner of the Torch River Provincial Forest, approximately 10 km north of Love, and contained pure jack pine (*Pinus banksiana*), with an understory of aspen (*Populus tremuloides*), green alder (*Alnus crispa*), bearberry (*Arctostaphylos uva-ursi*), and reindeer lichens (*Cladina spp.*) (EcoDynamics Consulting Ltd., 2007); the soil was characterized as a rapidly drained Orthic Eutric Brunisol with a medium sand texture. Two horizons were collected: the LFH to a depth of 6 cm (referred to as SK05-2LFH), and a mixture of the Ah-Bm horizon to a depth of approximately 25-30 cm (referred to as SK05-2-AB). The Ah horizon was very thin (2 cm) and was therefore collected together with the Bm horizon to minimize disturbance to the forest floor.

A.3 Chernozem Soils

Two soils were collected from Alberta, a reference soil and a corresponding soil contaminated with excessive salts. The site was located on a river floodplain terrace, with vegetation consisting of mostly smooth brome (*Bromus inermis* Leyss.) interspersed with rose (*Rosa* sp.), northern bedstraw (*Galium boreale* L.), and fireweed (*Epilobium angustifolium* L.). Adjacent areas contained forests dominated by aspen and white spruce (*Picea glauca*). The reference soil was characterized by a moderately-well drained Rego Dark Gray Chernozem, with the upper Ah horizon dominated by a silt loam texture, progressing to a very fine sand/loamy very fine sand to very sandy loam texture with depth (EcoDynamics Consulting Ltd., 2007). Two horizons were collected: the Ah horizon to a depth of approximately 11 cm (referred to as PR06-Ah (collected in 2006) or PR07-Ah (collected in 2007)), and the Ckgj horizon to a depth of approximately 30 cm (referred to as PR06-Ck or PR07-Ck).

The contaminated site was associated with an orphan gas well that had been impacted by a continuous discharge of salt-laden, mineral-rich groundwater, despite past capping attempts. The contaminated soil was collected from around the abandoned gas well, and lacked a natural soil profile due to past disturbances by drilling and remediation efforts; however, the mineral material was characterized by a very fine sandy loam texture, with localized deposits of loamy very fine sand and loam (EcoDynamics Consulting Ltd., 2007). Because of a lack of horizon differentiation, soil was collected to a depth of approximately 25 cm.

A.4 Gleysolic Soils

Similar to the Chernozem soils, two soils were also collected from Alberta: a reference soil and a corresponding soil contaminated with petroleum hydrocarbons. The site was located in a treed bog within an active oil and natural gas field that had been impacted by a crude oil spill in 1989; some remedial efforts were evident at the site (e.g., straw used as an oil-absorbent). The surficial geology of the collection area consisted of 20-30 cm of fibric and humic peat overlying

non-calcareous loamy to clayey lacustrine materials, characterized as poorly drained Rego Humic and Humic Luvic Gleysols, with loam to clay loam soil texture near the surface to underlying clay-rich deposits with depth (EcoDynamics Consulting Ltd., 2007). The majority of vegetation within the site consisted of black spruce (*Picea mariana*), with an understory dominated by peat (*Sphagnum* spp.) and haircap (*Polytrichum* spp.) mosses. In contrast, the contaminated site, where crude oil had pooled and concentrated, was mainly non-vegetated with haircap moss sporadically present.

Two horizons were collected from each site: the Of/Oh horizon to a depth of approximately 20-25 cm (referred to as SH06-Of/Oh (collected in 2006) or SH07-Of/Oh (collected in 2007)), and the Ahg horizon to a depth of approximately 25-35 cm from the surface (referred to as SH06-Ahg or SH07-Ahg).

APPENDIX B

The following presents the soil characterization of artificial soil used in the evaluation of the effect of phenanthrene on *Oppia nitens* (Chapter 4), including the raw bioaccumulation data and results of the uptake and elimination kinetic modeling.

Table B1. Physical and chemical characterization of artificial soil.

Parameter	Units	Method Reporting Limit	Artificial Soil
N-NH3 (Ammonia)	mg kg^{-1}	1	14
N-NO2 (Nitrite)	mg kg^{-1}	1	<1
N-NO3 (Nitrate)	mg kg^{-1}	1	6
pH			7.4
Sodium Adsorption Ratio (SAR)		0.01	0.26
Total Kjeldahl Nitrogen	%	0.01	0.07
Total Organic Carbon	%	0.01	5.45
Total Phosphorus	%	0.01	0.03
Buffer pH		2.0	
P (NaHCO3 Extractable)	mg kg^{-1}	2	9
K (NH4 Acetate Extractable)	mg kg^{-1}	10	11
Mg (NH4 Acetate Extractable)	mg kg^{-1}	10	77
Mn (Index)	Ind.	1	13
Zn (Index)	Ind.	1	12
Organic Matter	%	0.1	7.6
Na (NH4 Acetate Extractable)	mg kg^{-1}	10	44
Ca (NH4 Acetate Extractable)	mg kg^{-1}	100	2000
CEC K	Meq 100 g^{-1}	0.1	<0.1
CEC Mg	Meq 100 g^{-1}	0.1	0.6
CEC Ca	Meq 100 g^{-1}	0.1	10.0
CEC Na	Meq 100 g^{-1}	0.1	0.2
CEC Acid	Meq 100 g^{-1}	0.1	
CEC Total	Meq 100 g^{-1}	1	11
Base Saturation K	%	0.1	0.3
Base Saturation Mg	%	0.1	5.8
Base Saturation Ca	%	0.1	92.2
Base Saturation Na	%	0.1	1.8
Base Saturation Acid	%	0.1	
Base Saturation Total	%	0.1	100
Sand (>0.050 mm)	%	1	76
Silt (>0.002-0.050 mm)	%	1	12
Clay (<=0.002 mm)	%	1	12

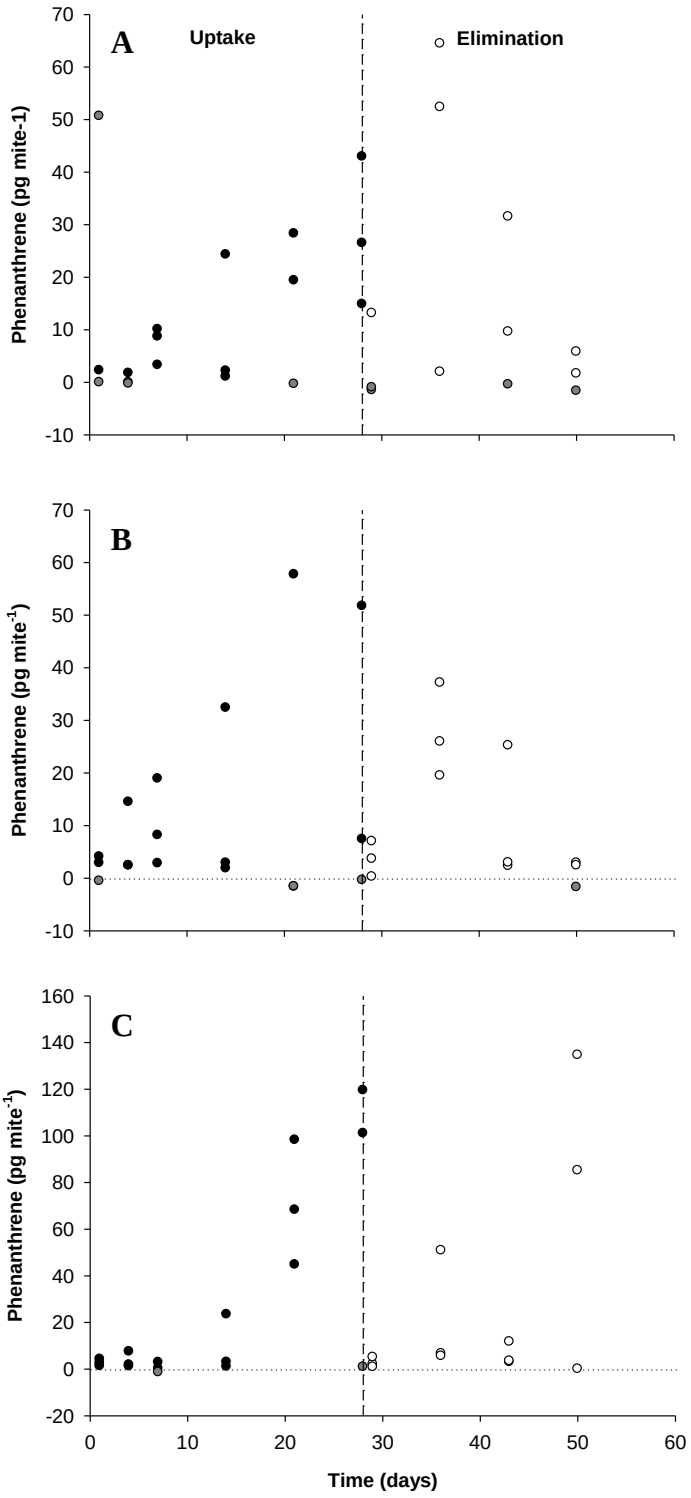

Fig. B1. The concentration of phenanthrene in *Oppia nitens* (pg mite^{-1}) upon exposure to nominal concentrations of 100 (A), 200 (B), and 400 (C) mg kg^{-1}artificial soil. The mites were exposed to phenanthrene for 28 days (referred to as the uptake phase), and then transferred to clean soil for an additional 21 days (referred to as the elimination phase). Data represent raw data, corrected for the control; grey circles are data points that were removed from the analysis.

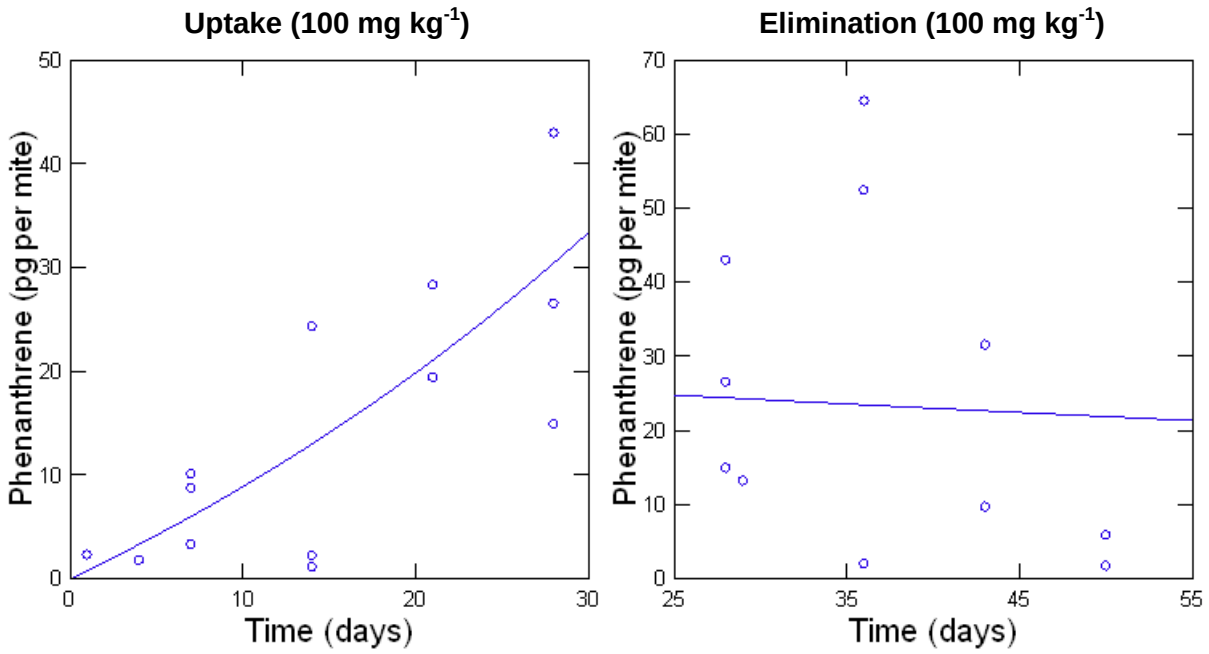

Fig. B2. Accumulation and elimination of phenanthrene by *Oppia nitens* exposed to artificial soil contaminated with 100 mg kg^{-1}.

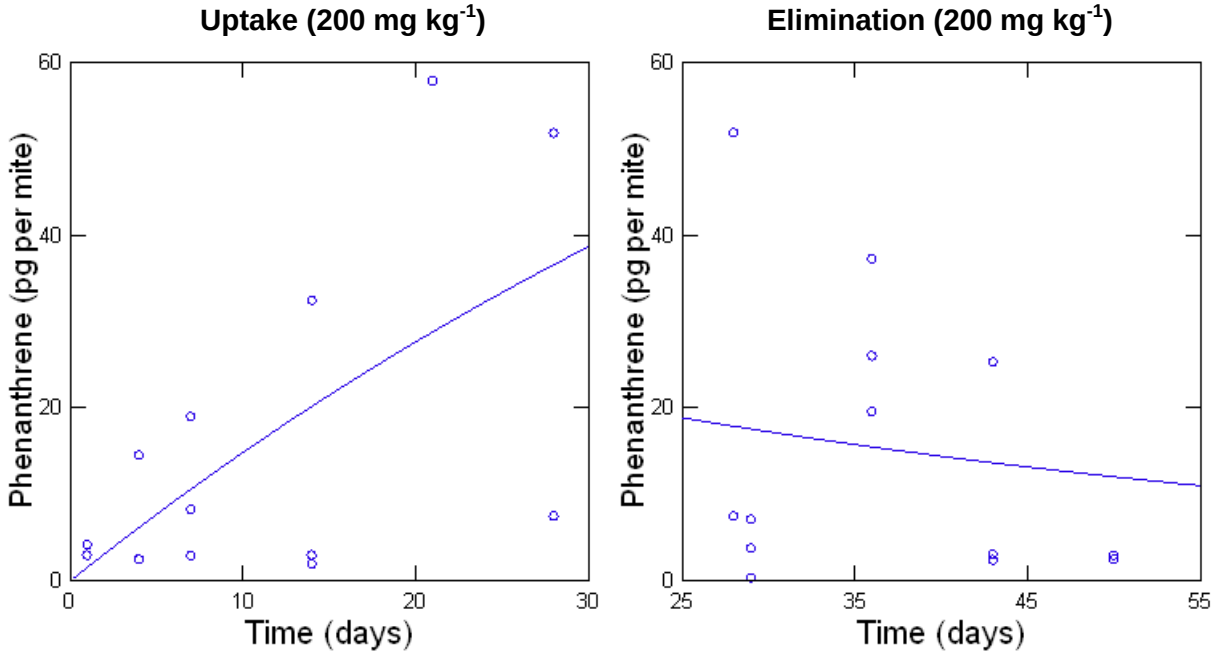

Fig. B3. Accumulation and elimination of phenanthrene by *Oppia nitens* exposed to artificial soil contaminated with 200 mg kg^{-1}.

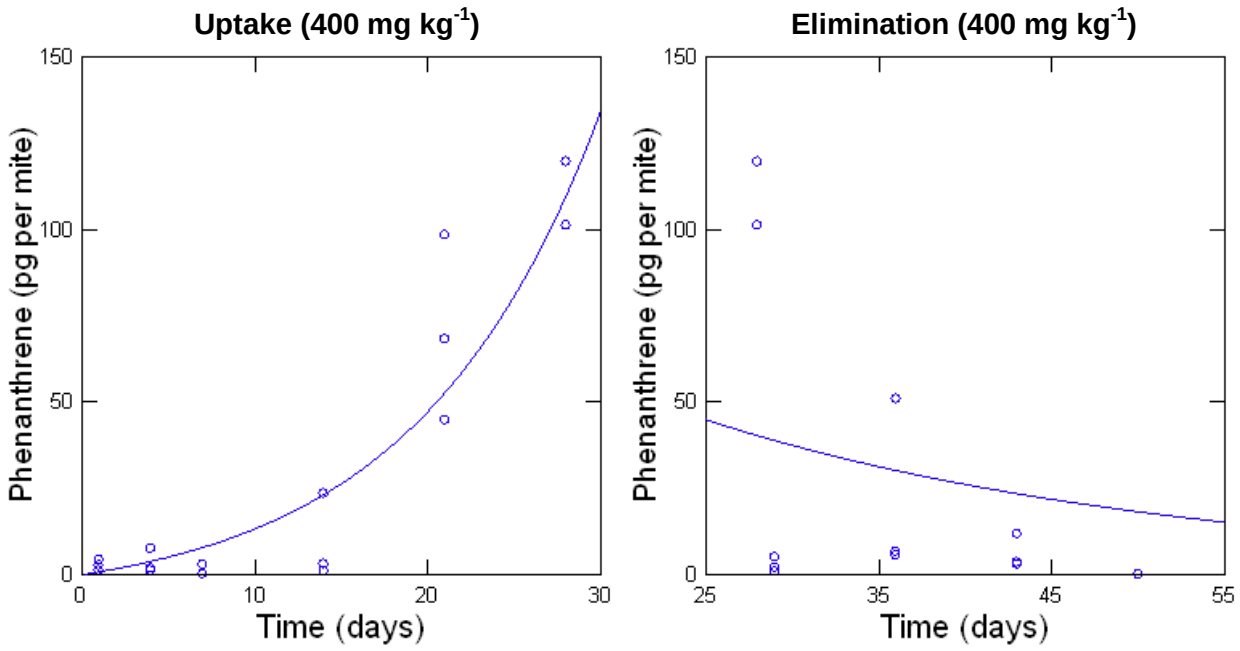

Fig. B4. Accumulation and elimination of phenanthrene by *Oppia nitens* exposed to artificial soil contaminated with 400 mg kg^{-1}.